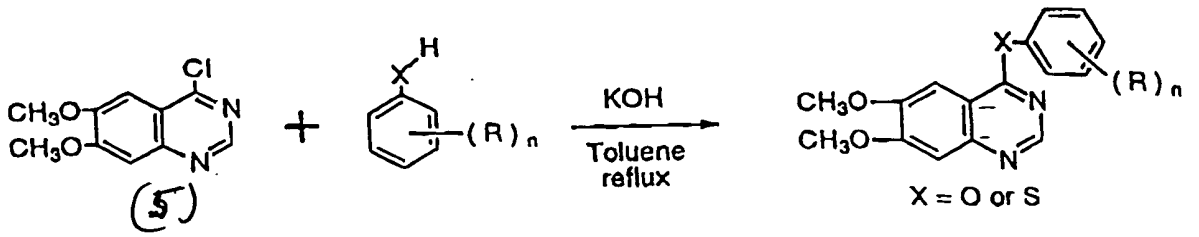
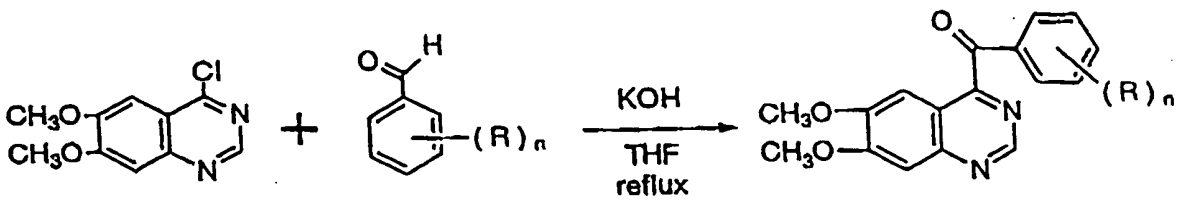
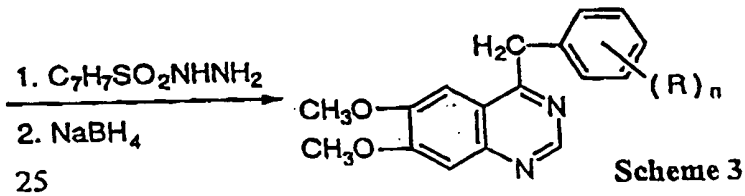
Figure 1







Scheme 3

Fig 1

Figure 6

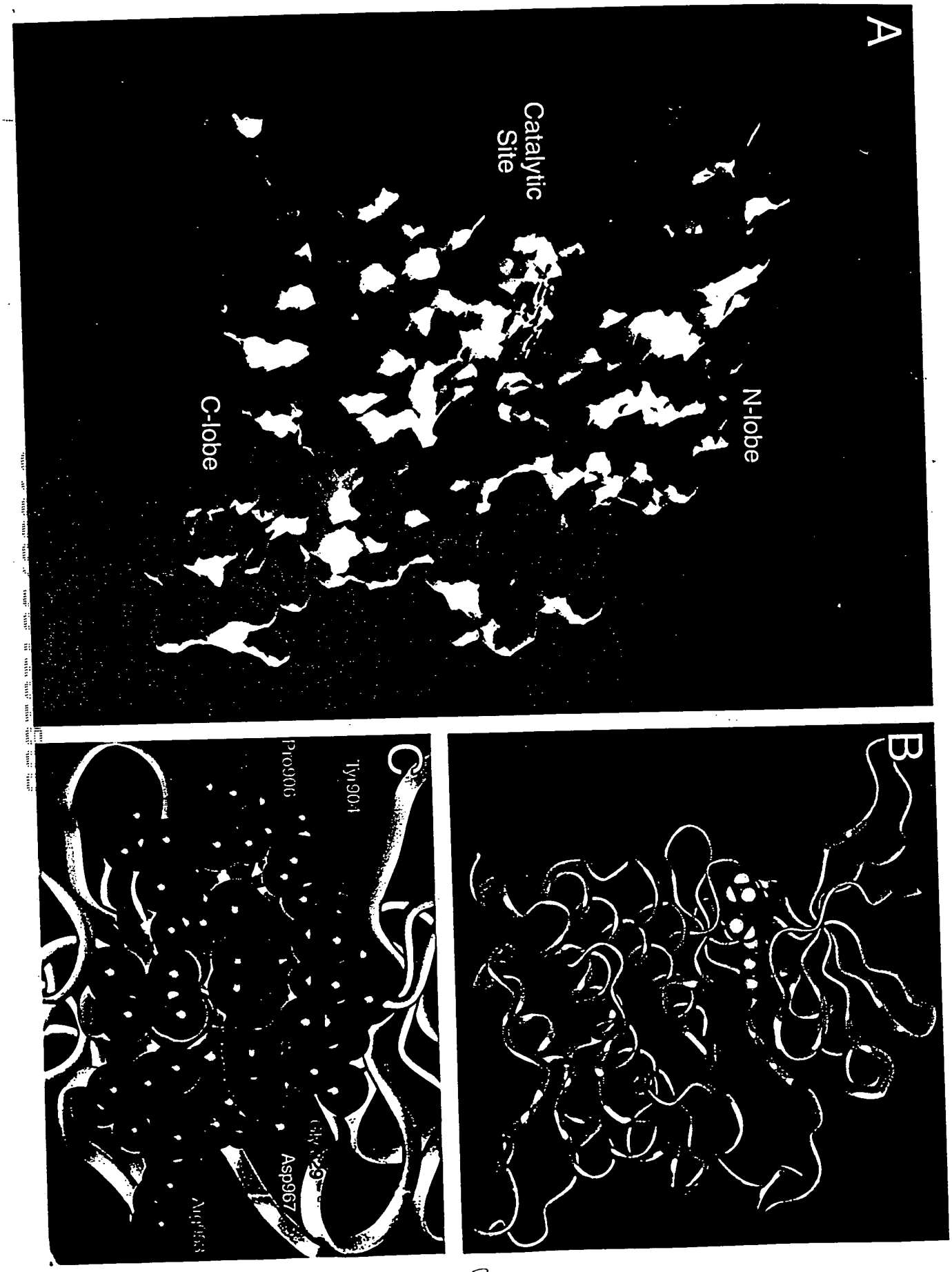
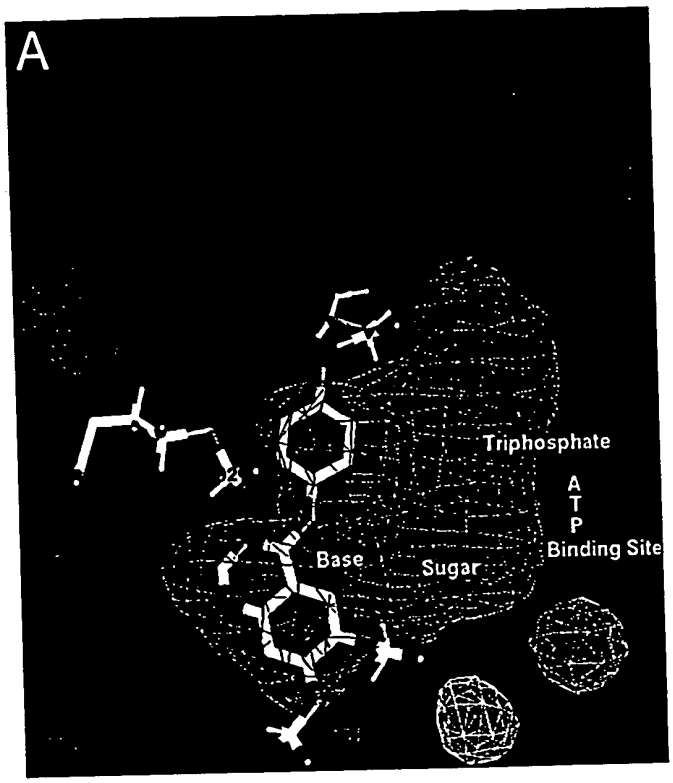
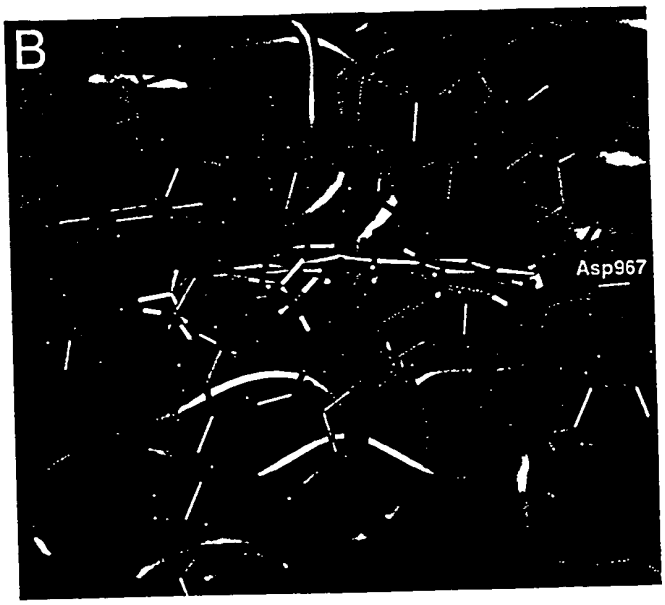
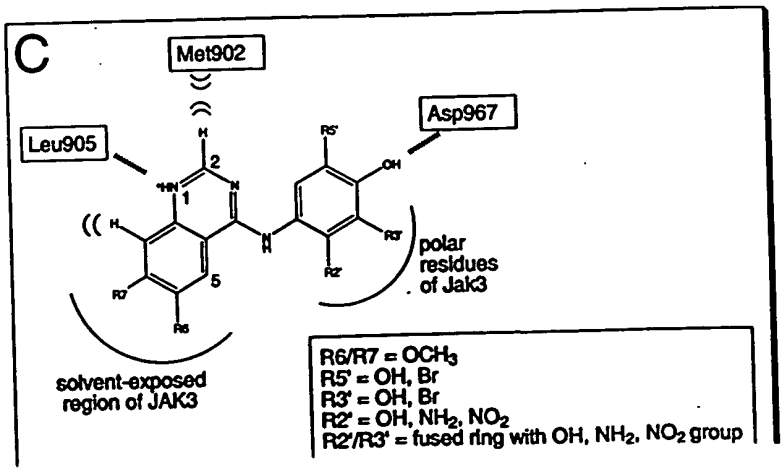

A
N-lobe
Catalytic Site
C-lobe
B
C
Tyr904
Pro906
Gly
Asp907
Arg

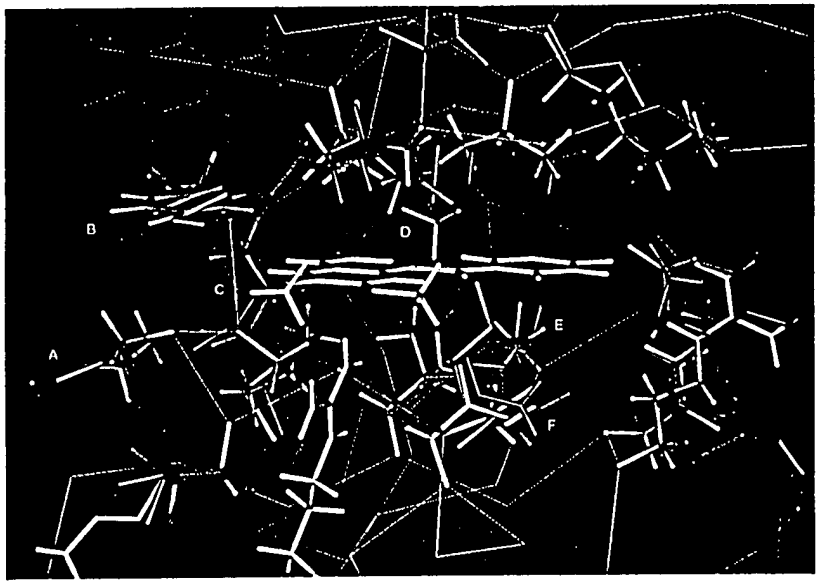
Figure 3


A
Triphosphate
A
T
P
Binding Site
Base
Sugar


B
Asp967


C
Met902
Leu905
Asp967
polar
residues
of Jak3
solvent-exposed
region of JAK3
R6/R7 = OCH3
R5' = OH, Br
R3' = OH, Br
R2' = OH, NH2, NO2
R2'/R3' = fused ring with OH, NH2, NO2 group

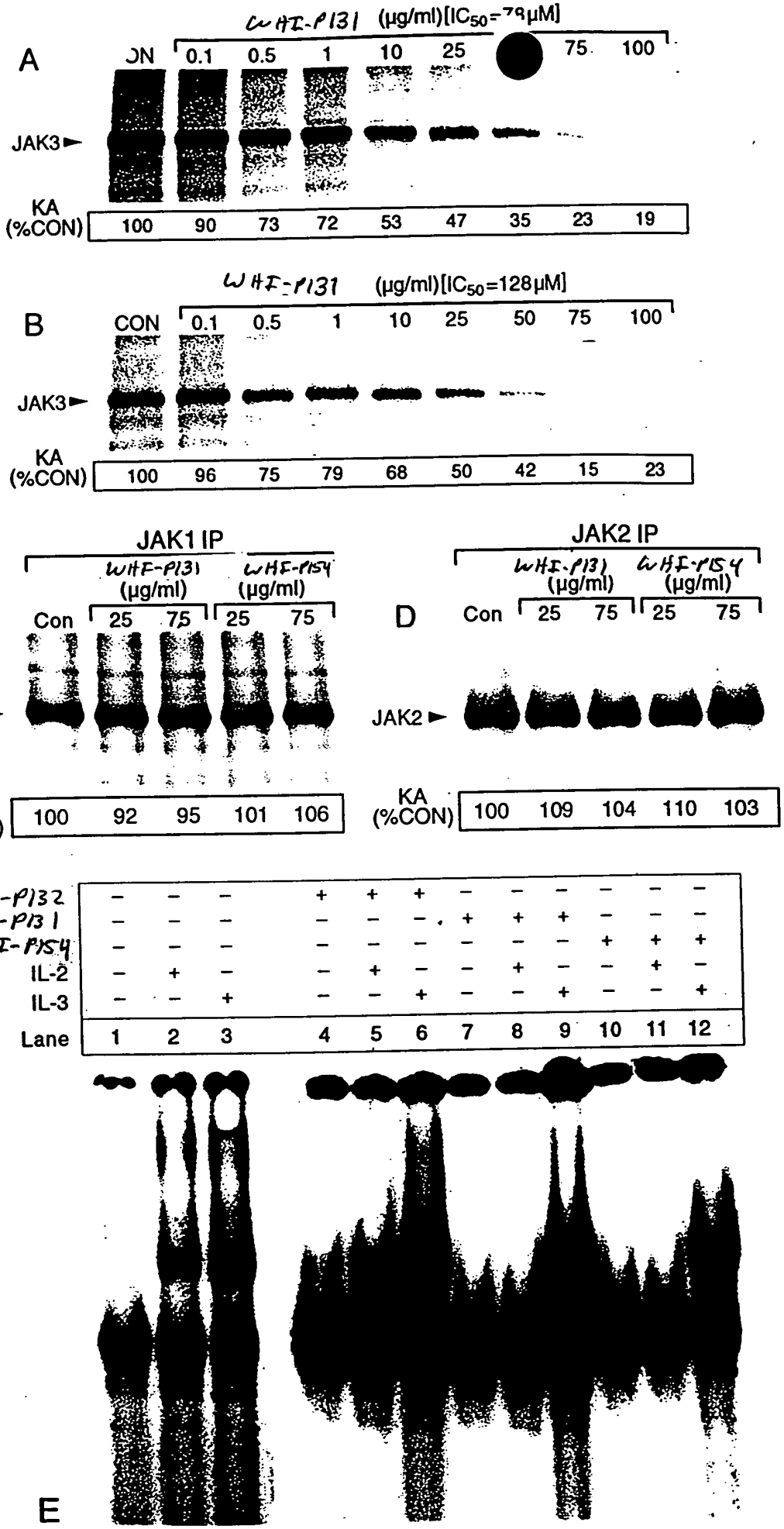
A

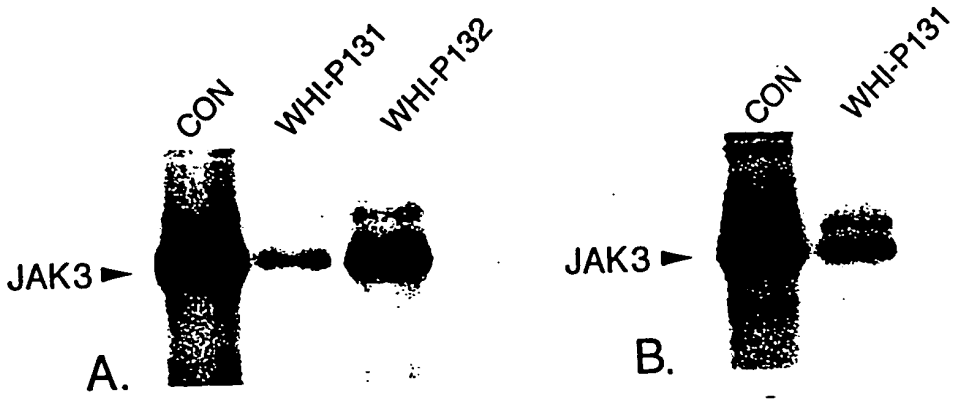


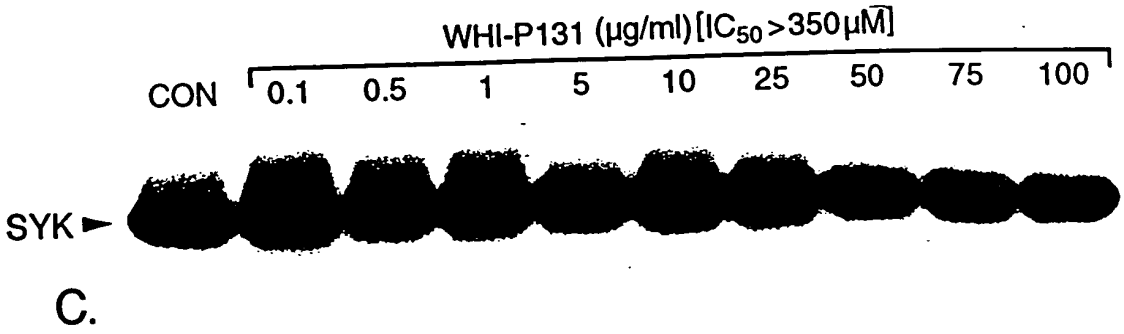
B

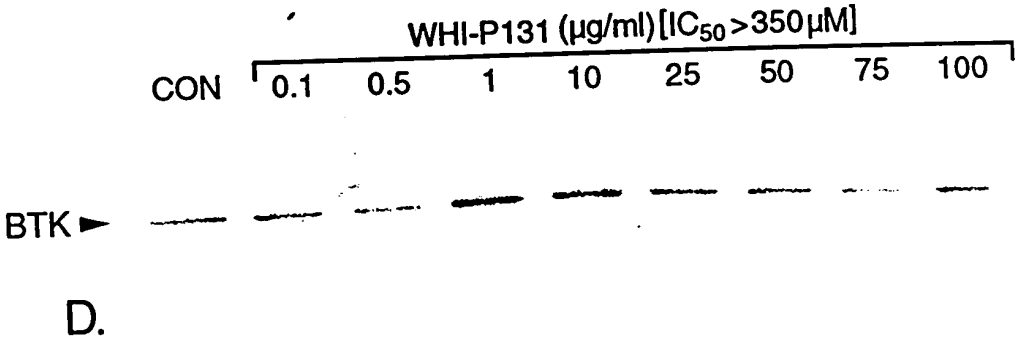
Protein Tyrosine Kinase	Residue at Region A	Residue at Region B	Residue at Region C	Residue at Region D	Residue at Region E	Residue at Region F
JAK3	Pro906	Tyr904	Leu905	Met902	Ala966	Asp912
JAK2	Pro933	Tyr931	Leu932	Met929	Gly993	Asp939
JAK1	Pro948	Phe946	Leu947	Met944	Gly1008	Glu954
BTK	Ala478	Tyr476	Met477	Thr474	Ser538	Asn484
SYK	Glu342	Met340	Ala341	Met338	Ser403	Lys348
HCK	Ala342	Phe340	Met341	Ala342	Ala403	Asp348
LYN	Ala323	Tyr321	Met322	Ala323	Ala384	Asp329
IRK	Ala1080	Leu1078	Met1079	Met1076	Gly1149	Ser1086

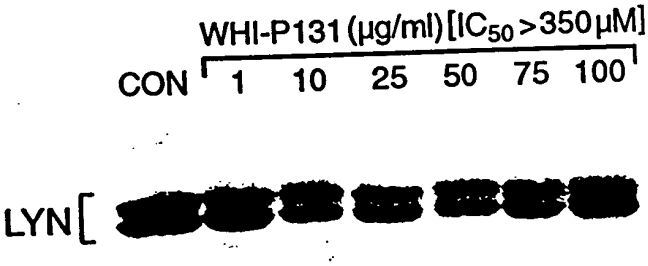
Figure 4

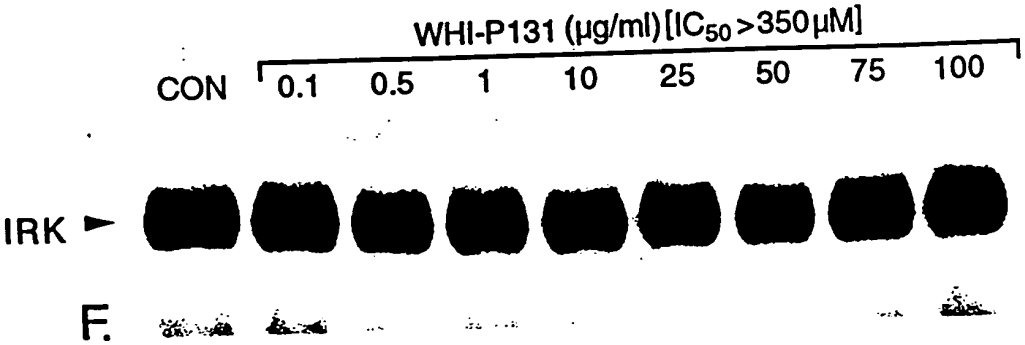

WHI-P131 (μg/ml)[IC50=78μM]
A
CON
0.1
0.5
1
10
25
75
100
JAK3
KA (%CON)
100 90 73 72 53 47 35 23 19
WHI-P131 (μg/ml)[IC50=128μM]
B
CON
0.1
0.5
1
10
25
50
75
100
JAK3
KA (%CON)
100 96 75 79 68 50 42 15 23
JAK1 IP
WHI-P131 (μg/ml)
WHI-P154 (μg/ml)
C
Con
25
75
25
75
JAK1
KA (%CON)
100 92 95 101 106
JAK2 IP
WHI-P131 (μg/ml)
WHI-P154 (μg/ml)
D
Con
25
75
25
75
JAK2
KA (%CON)
100 109 104 110 103
WHI-P132
WHI-P131
WHI-P154
IL-2
IL-3
Lane
1 2 3 4 5 6 7 8 9 10 11 12
E

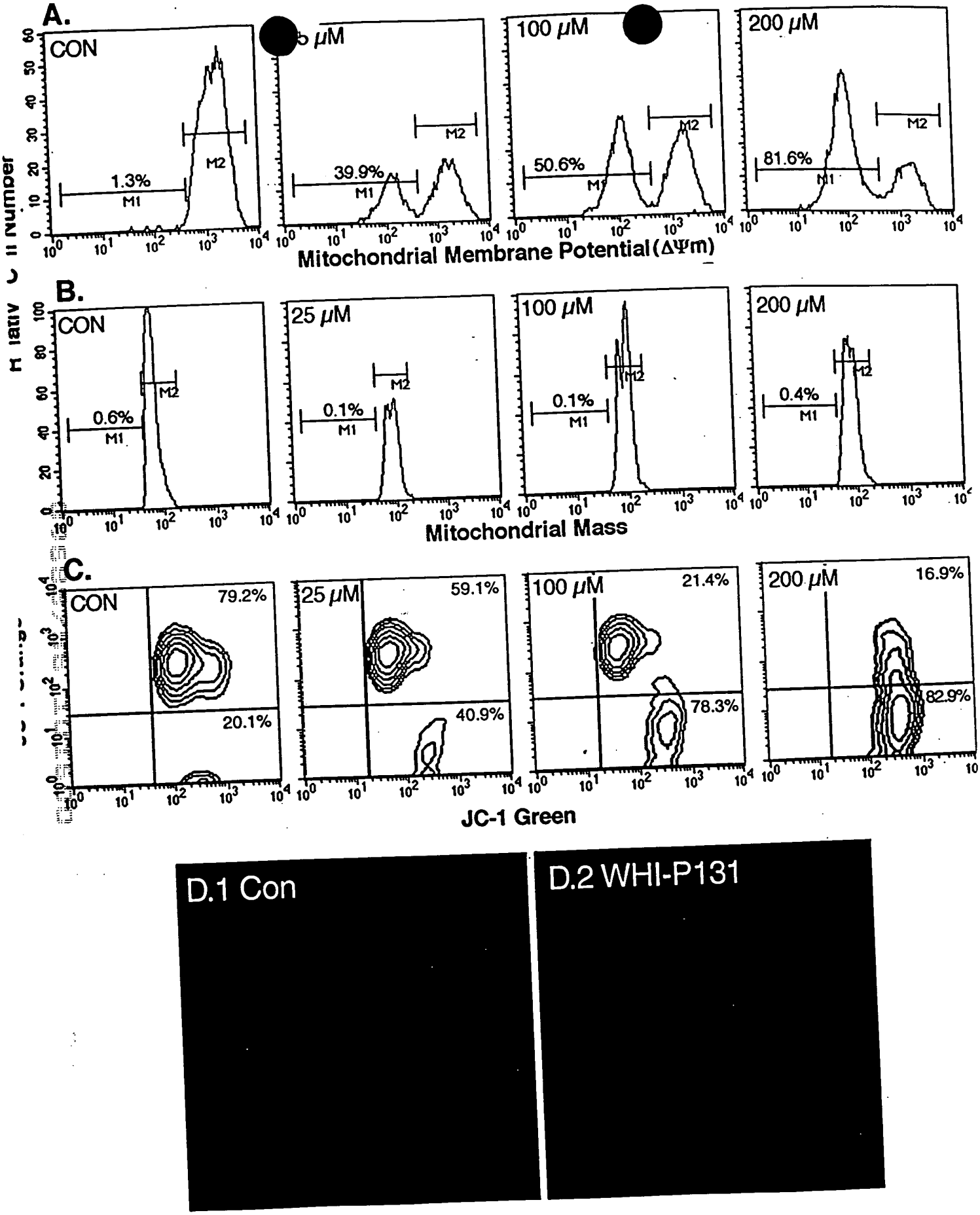

CON
WHI-P131
WHI-P132
JAK3
A.
CON
WHI-P131
JAK3
B.

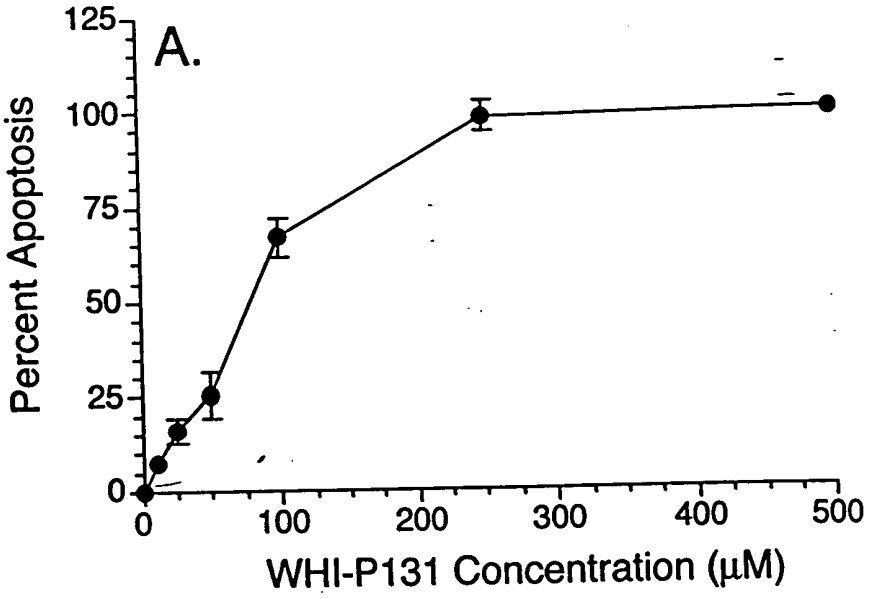

WHI-P131 (μg/ml) [IC50 >350μM]
CON
0.1
0.5
1
5
10
25
50
75
100
SYK
C.

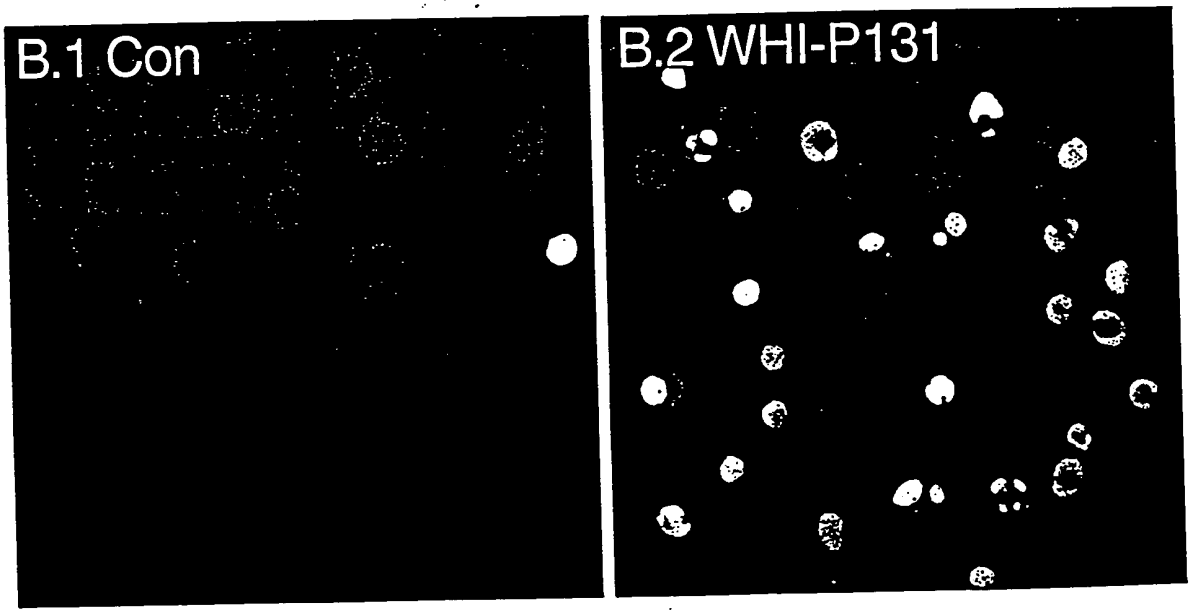

WHI-P131 (μg/ml) [IC50 >350μM]
CON
0.1
0.5
1
10
25
50
75
100
BTK
D.


WHI-P131 (μg/ml) [IC50 >350μM]
CON
1
10
25
50
75
100
LYN
E.


WHI-P131 (μg/ml) [IC50 >350μM]
CON
0.1
0.5
1
10
25
50
75
100
IRK
F.

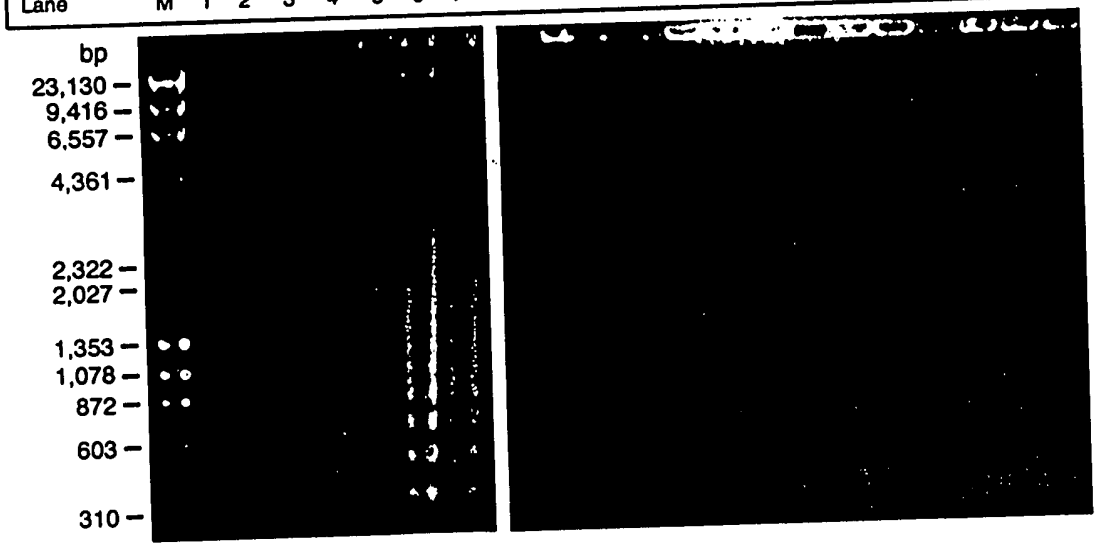

A.
CON
25 μM
100 μM
200 μM
1.3%
39.9%
50.6%
81.6%
M1
M2
Mitochondrial Membrane Potential (ΔΨm)
B.
CON
25 μM
100 μM
200 μM
0.6%
0.1%
0.1%
0.4%
Mitochondrial Mass
C.
CON
25 μM
100 μM
200 μM
79.2%
20.1%
59.1%
40.9%
21.4%
78.3%
16.9%
82.9%
JC-1 Green
D.1 Con
D.2 WHI-P131


A.
Percent Apoptosis
125
100
75
50
25
0
0
100
200
300
400
500
WHI-P131 Concentration (μM)


B.1 Con
B.2 WHI-P131

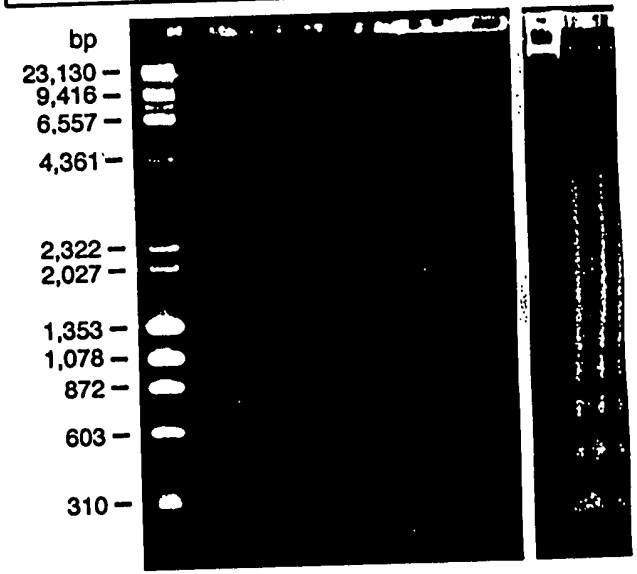
A

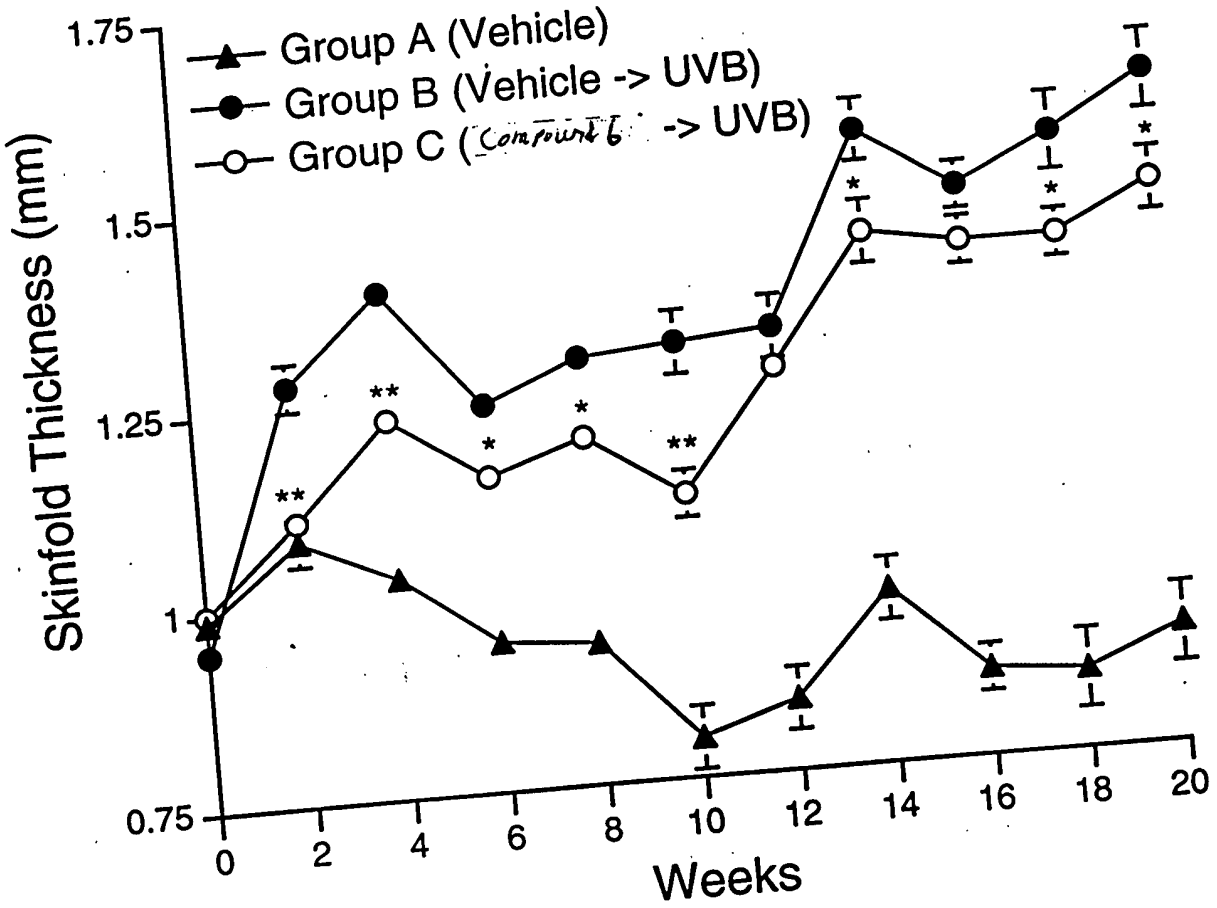
WHI-P79	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0	1	3
WHI-P132	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0	1	3	–	–	–
WHI-P112	–	–	–	–	–	–	–	–	–	–	–	–	0	1	3	–	–	–	–	–	–	–
WHI-P111	–	–	–	–	–	–	–	–	–	0	1	3	–	–	–	–	–	–	–	–	–	–
WHI-P131	–	–	–	–	–	–	1	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–
WHI-P258	–	0	1	3	10	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Lane	M	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21



B

LCl;19	–	–	–	–	–	–	–	–	+	+
SQ20B	–	–	–	–	–	+	+	+	–	–
M24-MET	–	–	+	+	+	–	–	–	–	–
WHI-P131	–	–	0	1	3	0	1	3	0	1
Lane	M	1	2	3	4	5	6	7	8	9	10




Group A (Vehicle)
Group B (Vehicle -> UVB)
Group C (Compound 6 -> UVB)
Skinfold Thickness (mm)
1.75
1.5
1.25
1
0.75
0
2
4
6
8
10
12
14
16
18
20
Weeks

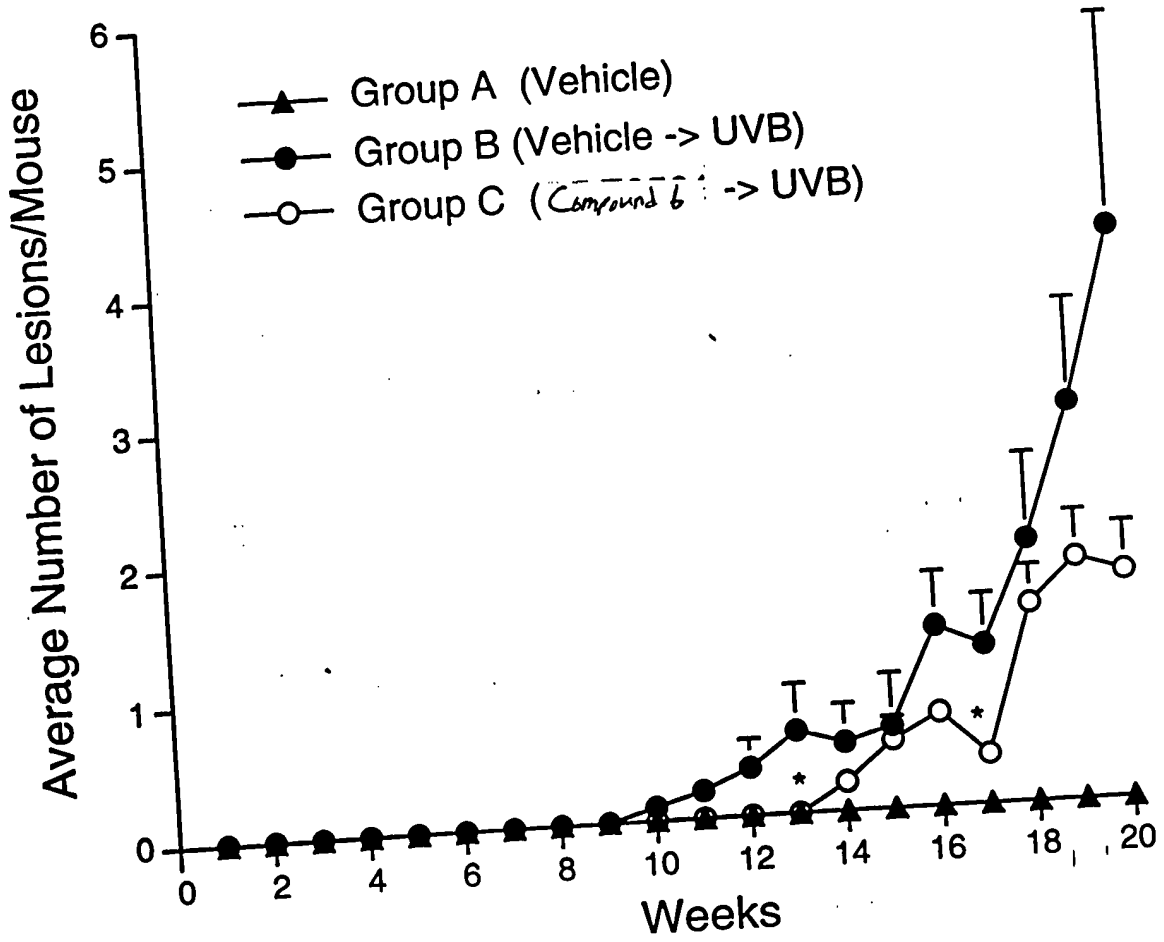

Group A (Vehicle)
Group B (Vehicle -> UVB)
Group C (Compound b -> UVB)
Average Number of Lesions/Mouse
Weeks
0
1
2
3
4
5
6
0
2
4
6
8
10
12
14
16
18
20
*
*

Figure 1C

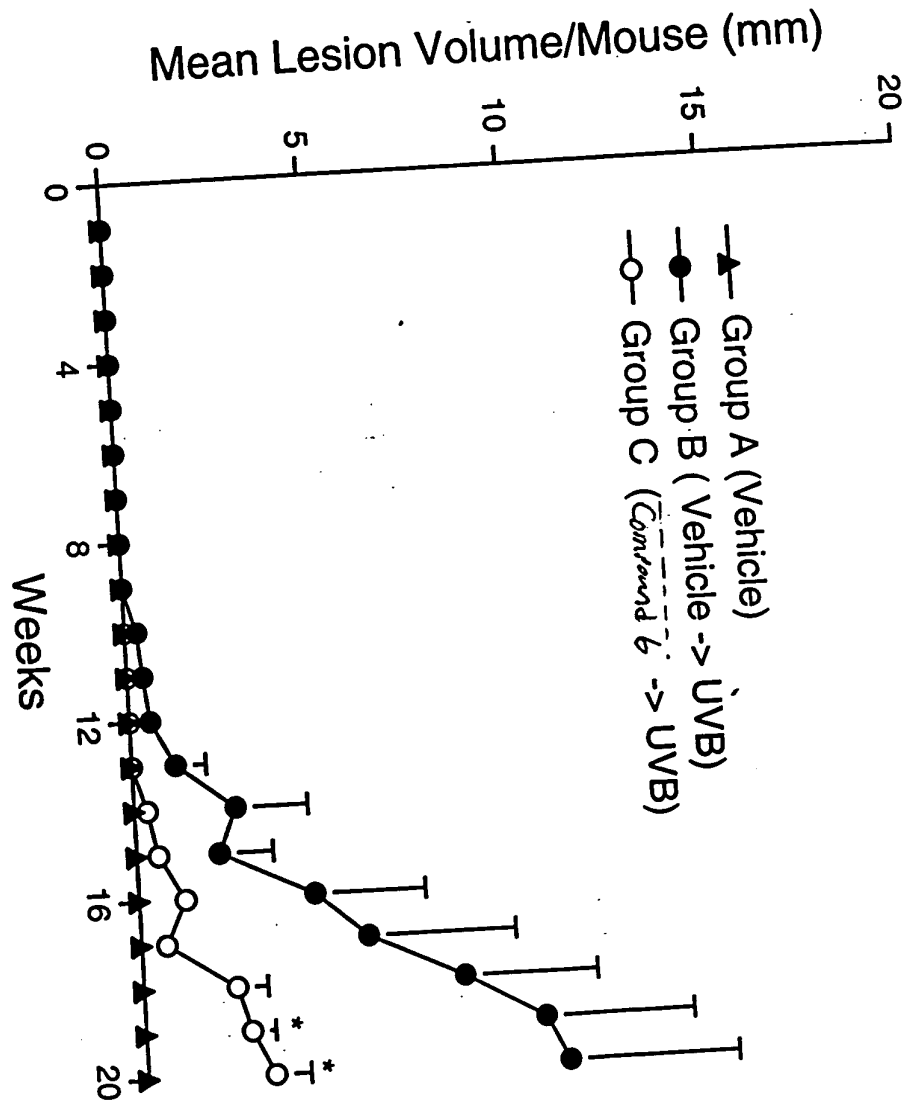
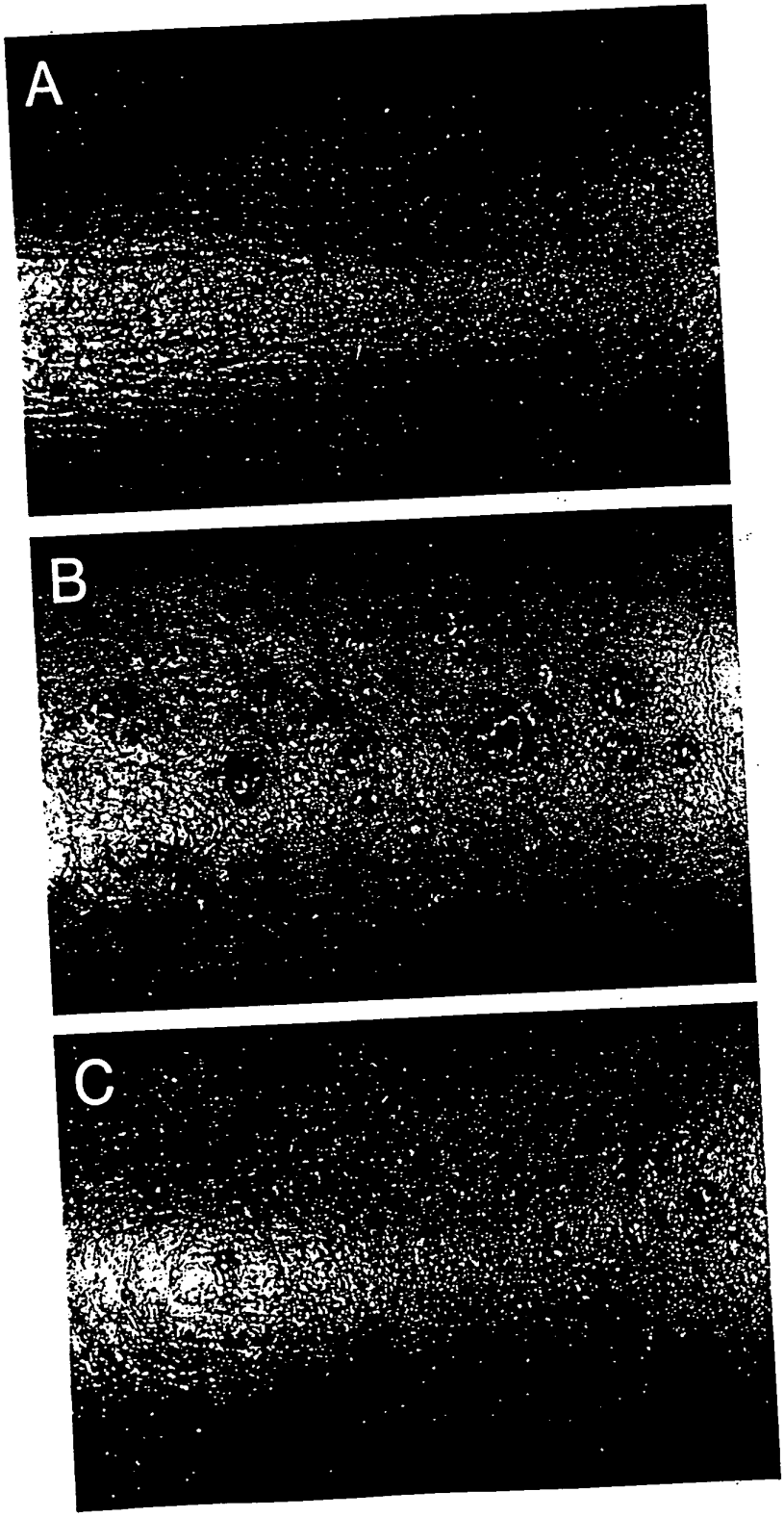

Mean Lesion Volume/Mouse (mm)
0
5
10
15
20
Weeks
0
4
8
12
16
20
Group A (Vehicle)
Group B (Vehicle -> UVB)
Group C (Compound 6 -> UVB)
*
*

Figure 15



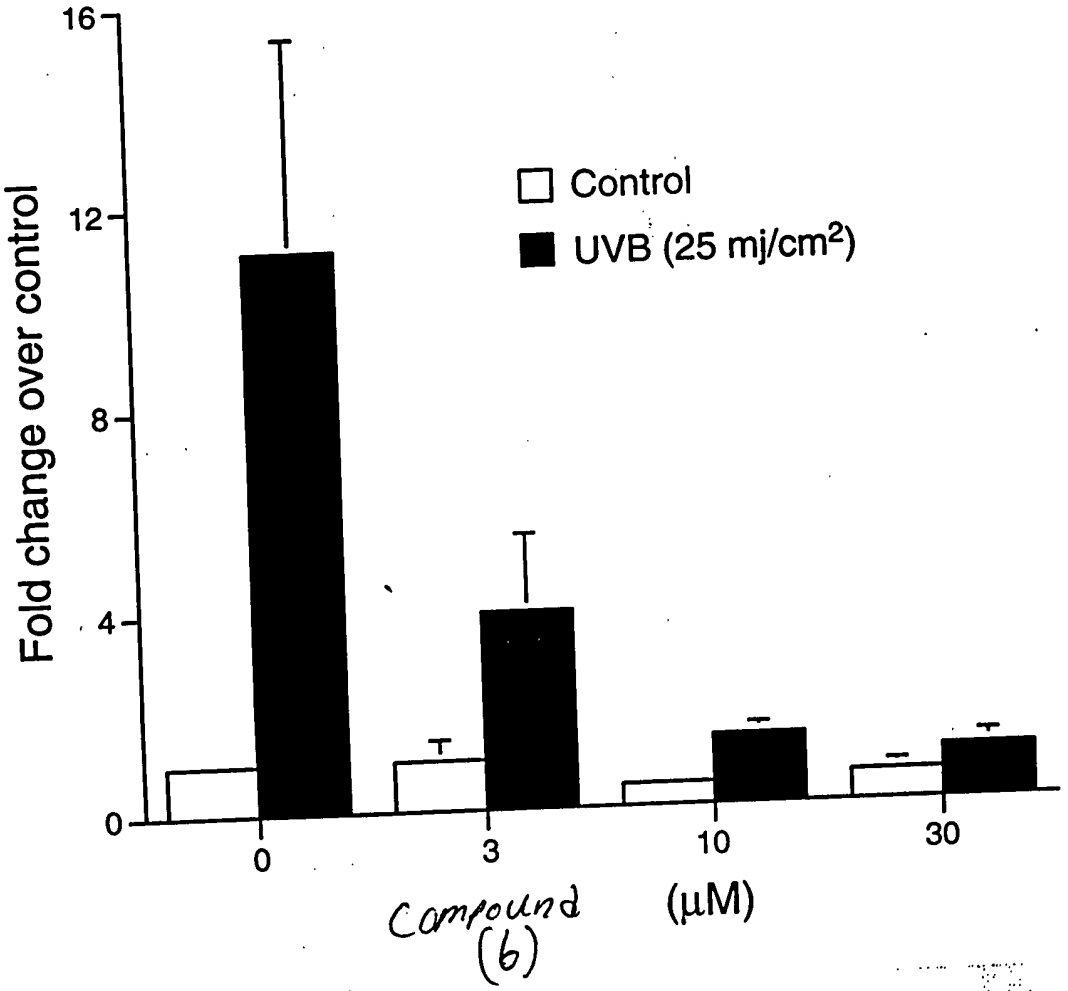
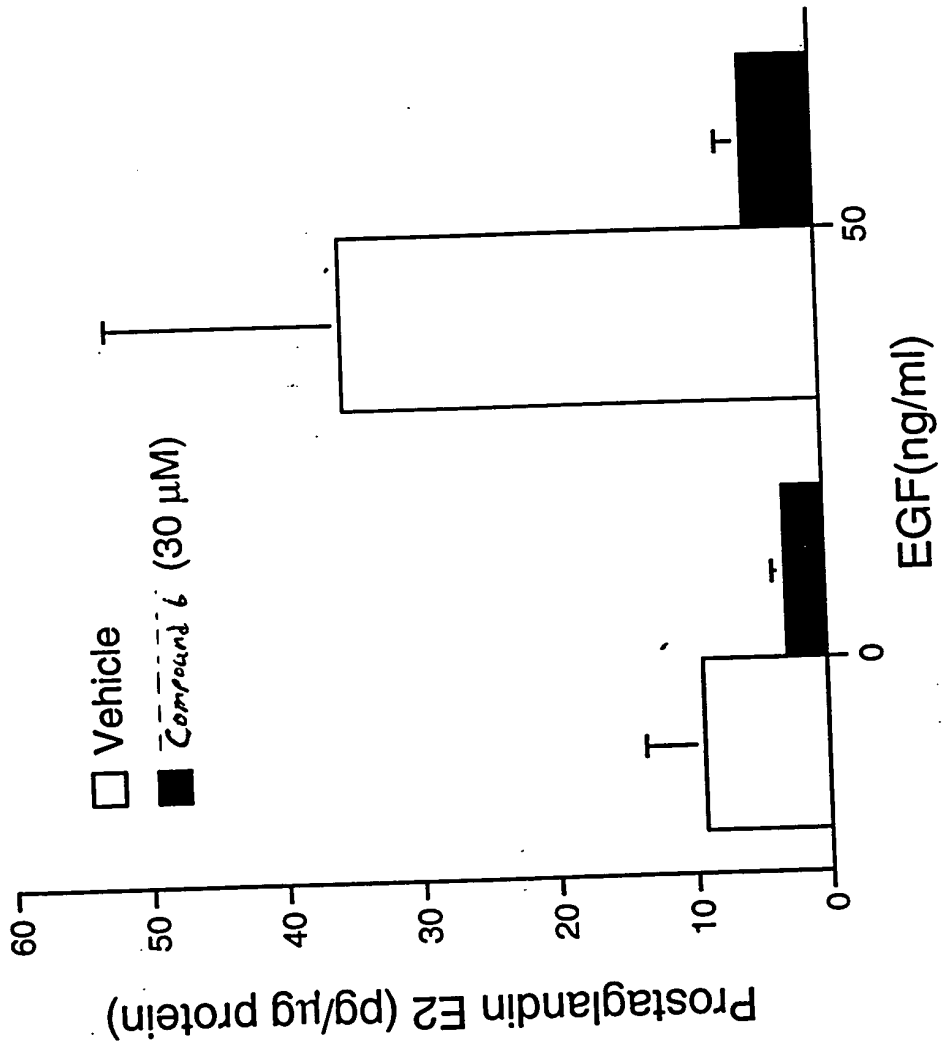
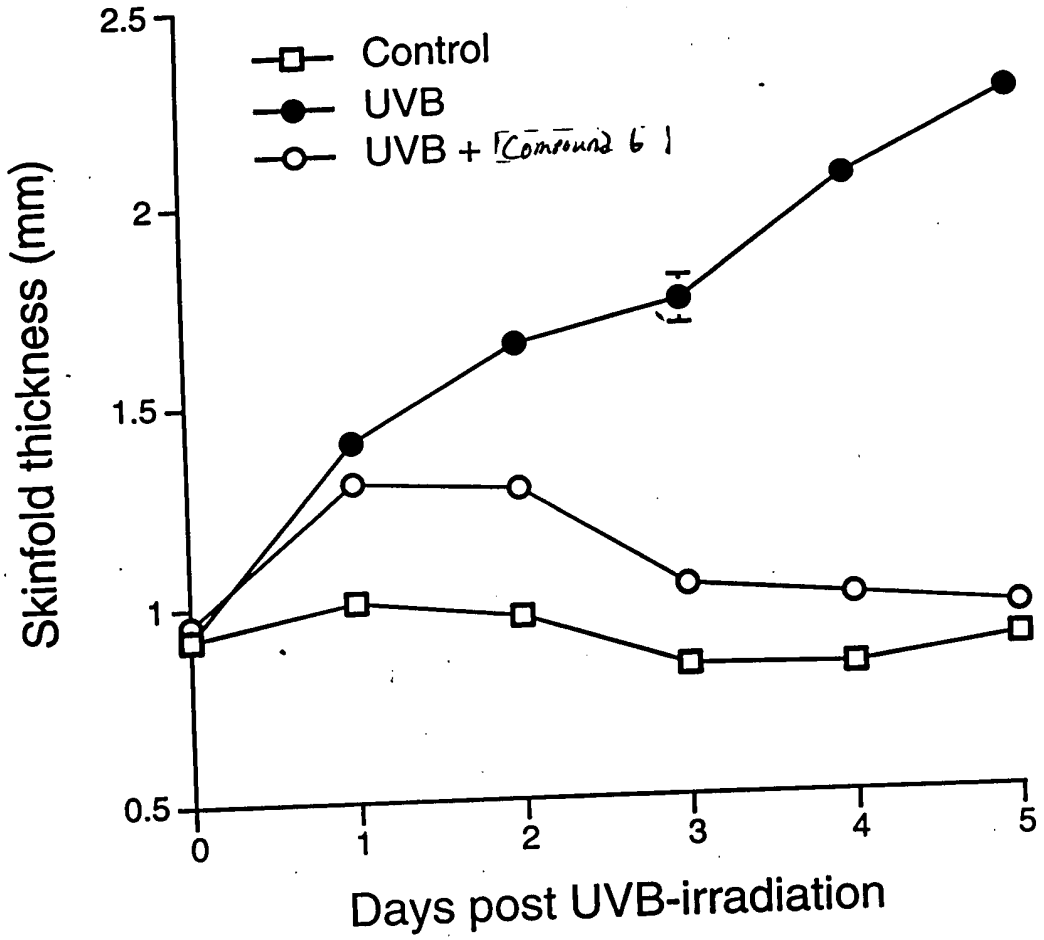
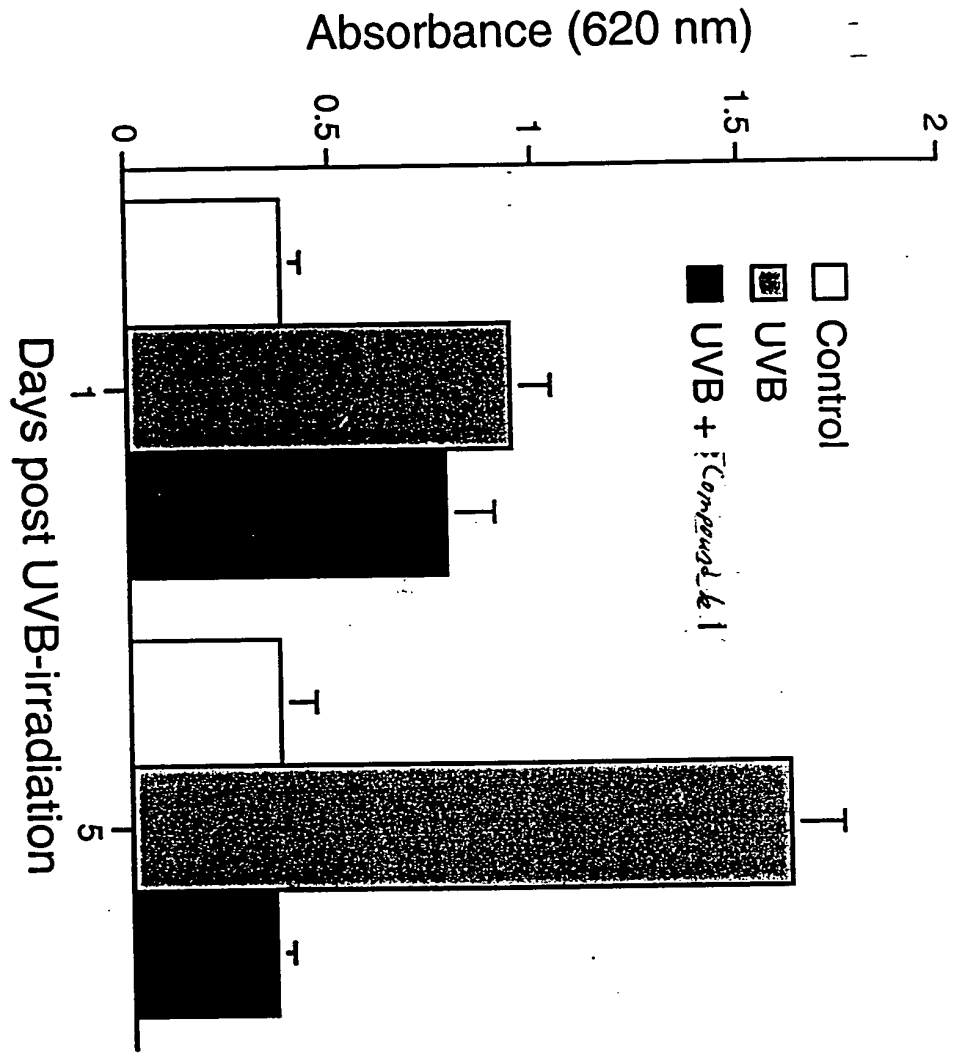

Fold change over control
16
12
8
4
0
Control
UVB (25 mj/cm²)
0
3
10
30
Compound (6)
(μM)

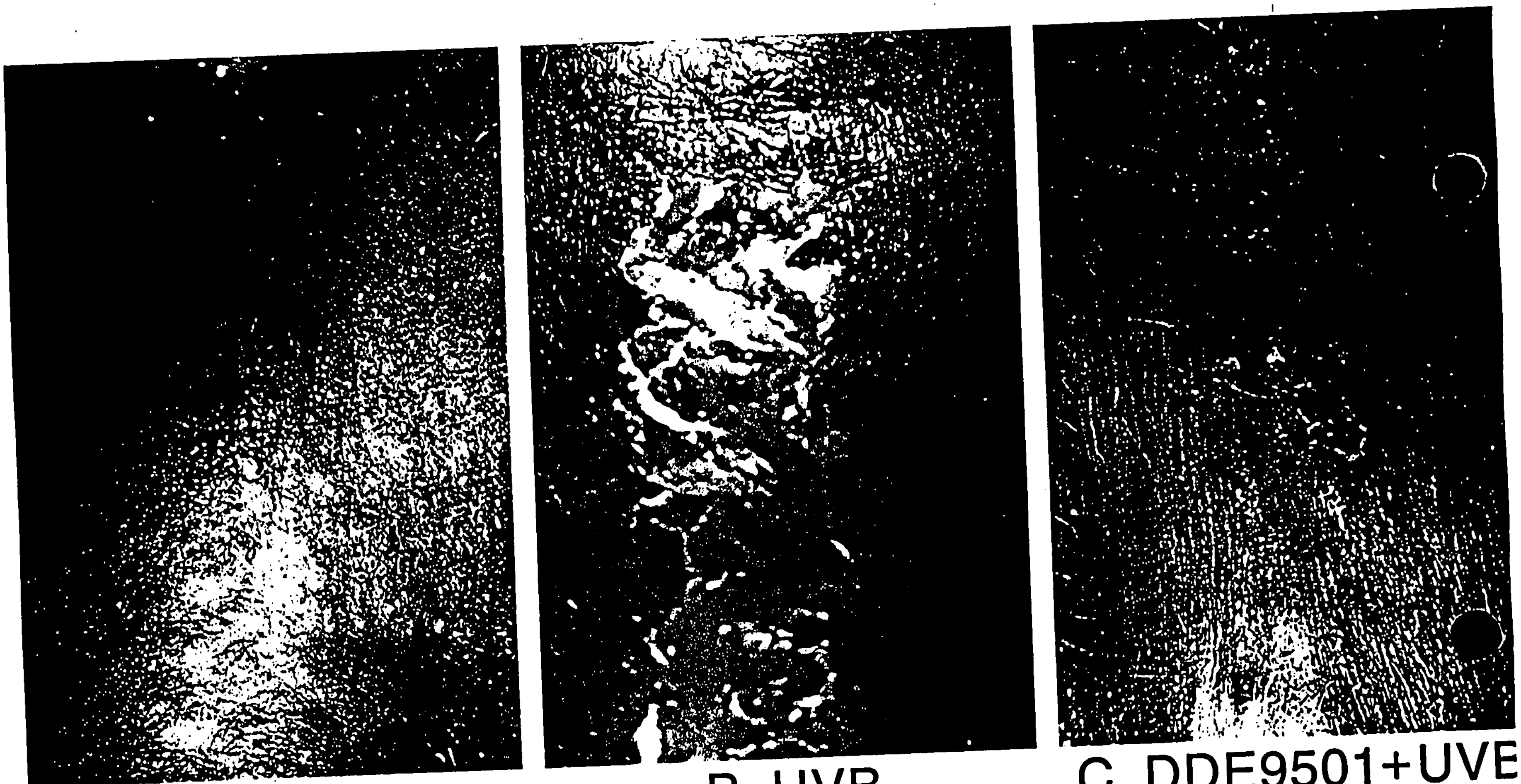
Figure 17

Figure 15

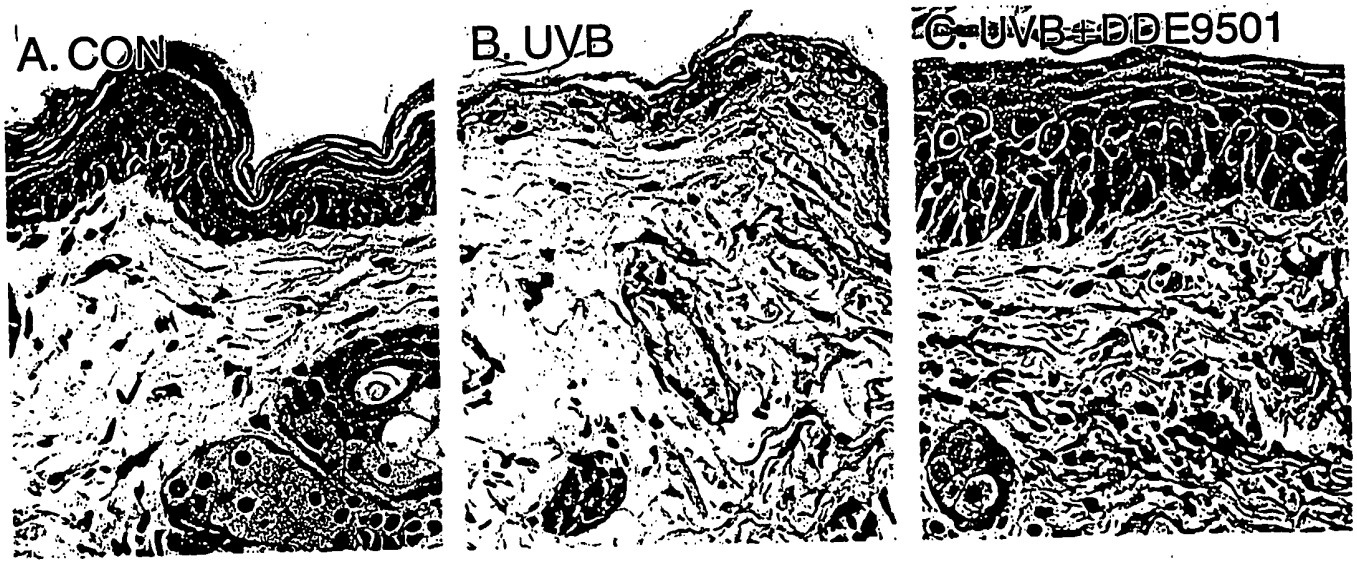

Prostaglandin E2 (pg/µg protein)
60
50
40
30
20
10
0
Vehicle
Compound i (30 µM)
T
T
T
0
50
EGF(ng/ml)


Control
UVB
UVB + [Compound 6]
Skinfold thickness (mm)
2.5
2
1.5
1
0.5
0
1
2
3
4
5
Days post UVB-irradiation


Absorbance (620 nm)
0
0.5
1
1.5
2
Control
UVB
UVB + Compound 4.1
Days post UVB-irradiation
1
5



A. Control   B. UVB   C. DDE9501+UVE


A. CON
B. UVB
C. UVB+DDE9501

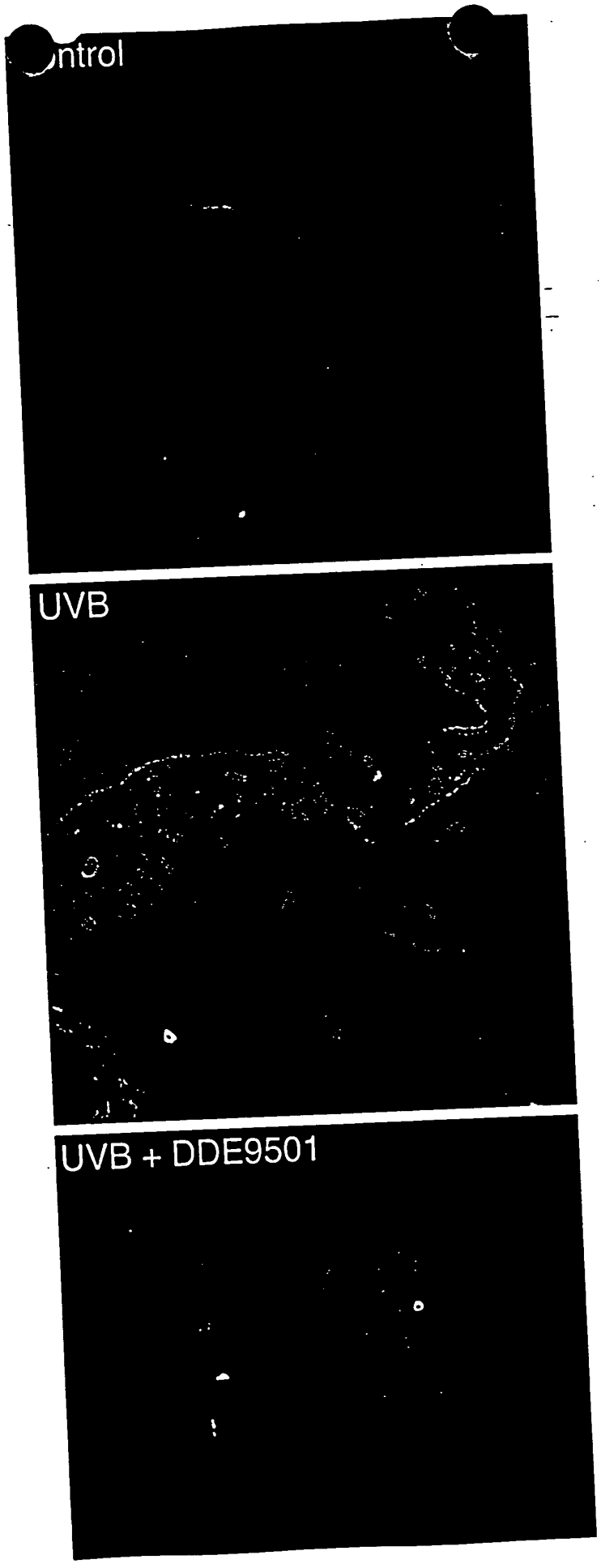

ntrol
UVB
UVB + DDE9501

Figure 21

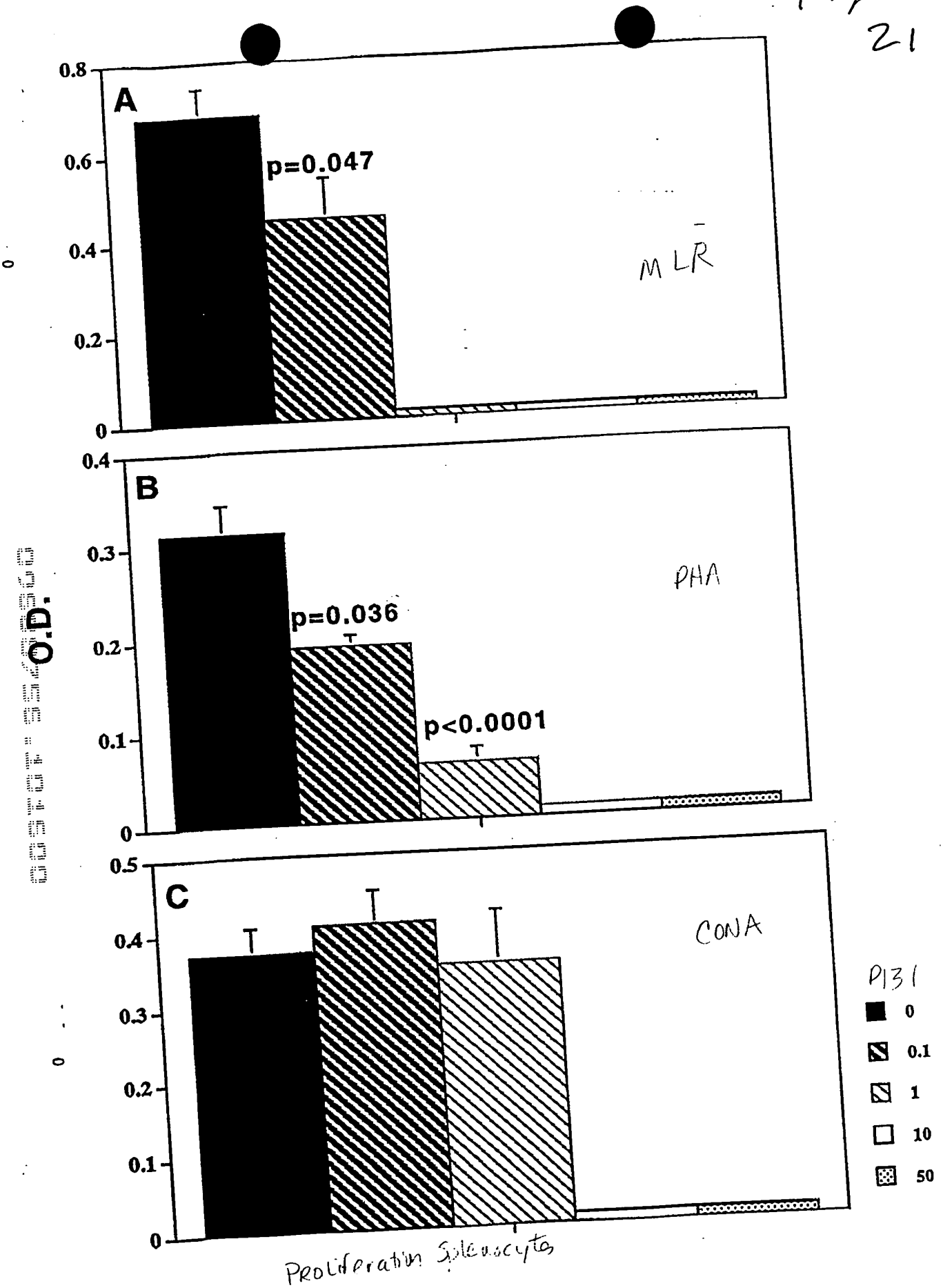
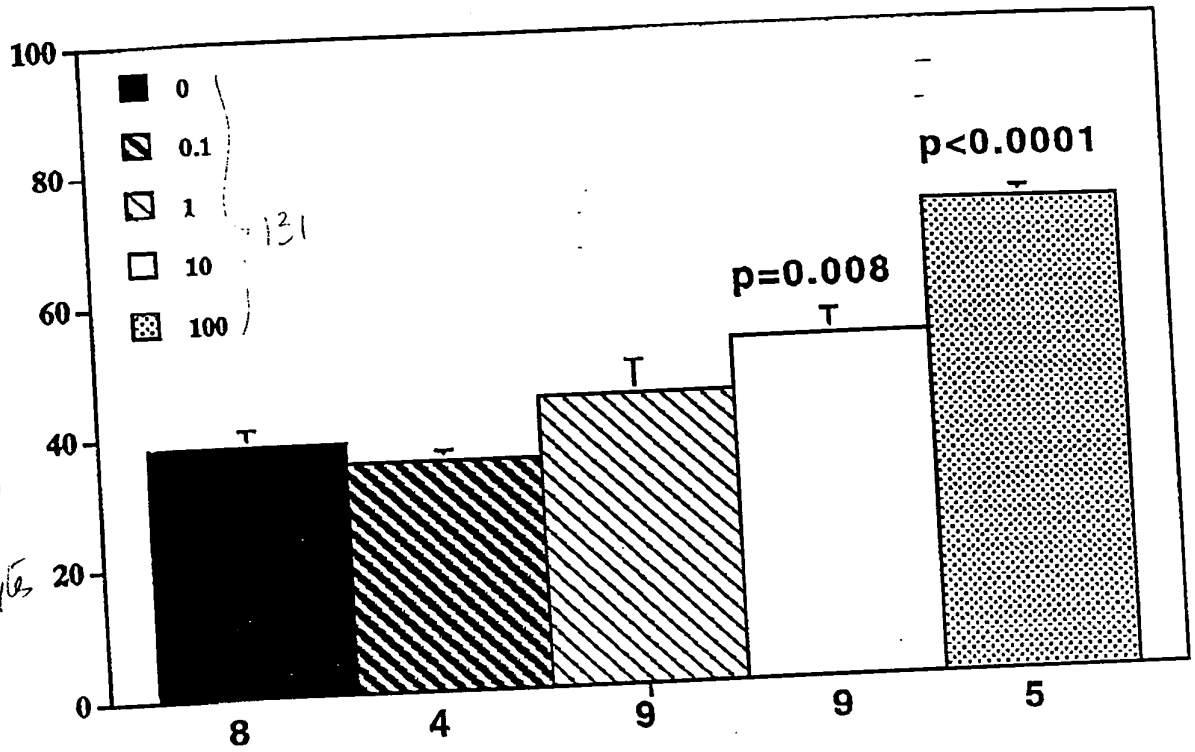
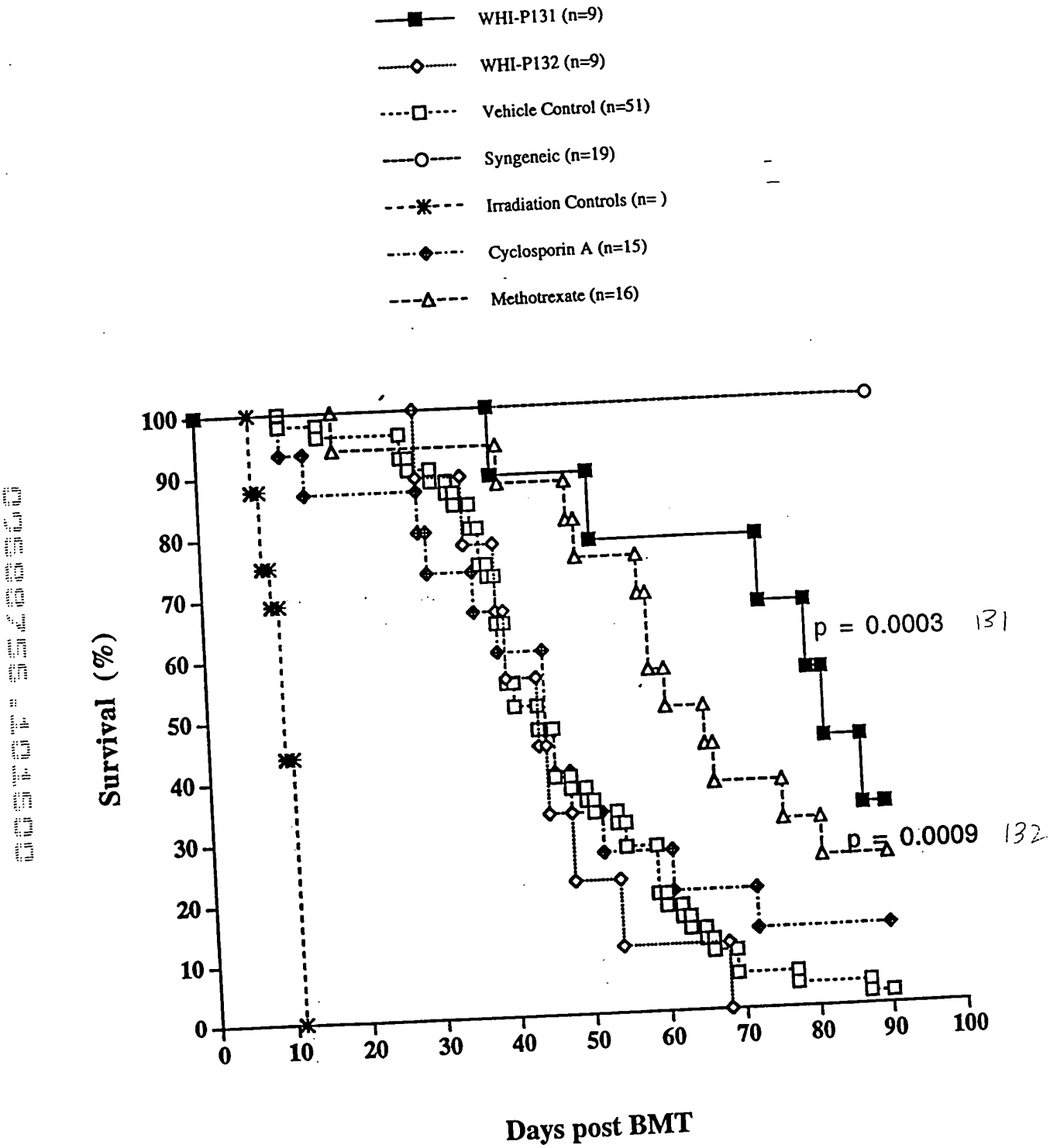

A
0.8
0.6
0.4
0.2
0
p=0.047
MLR
B
0.4
0.3
0.2
0.1
0
p=0.036
p<0.0001
PHA
C
0.5
0.4
0.3
0.2
0.1
0
CONA
O.D.
Proliferation Splenocytes
PI31
0
0.1
1
10
50

Figure 11



spleen cells

P131 promotes apoptosis in spleen cells



131, 132 ↑ survival

Figure 24

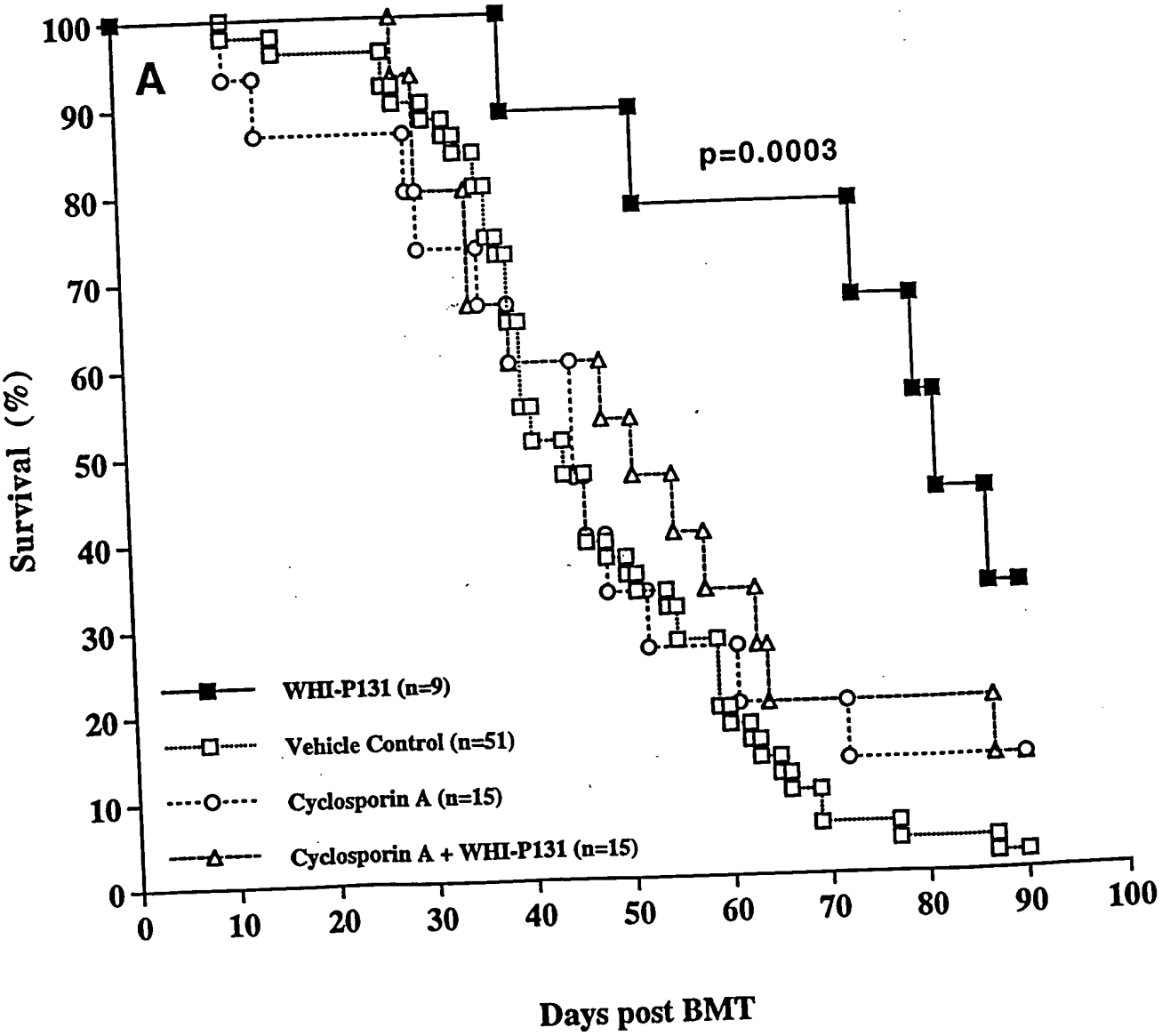
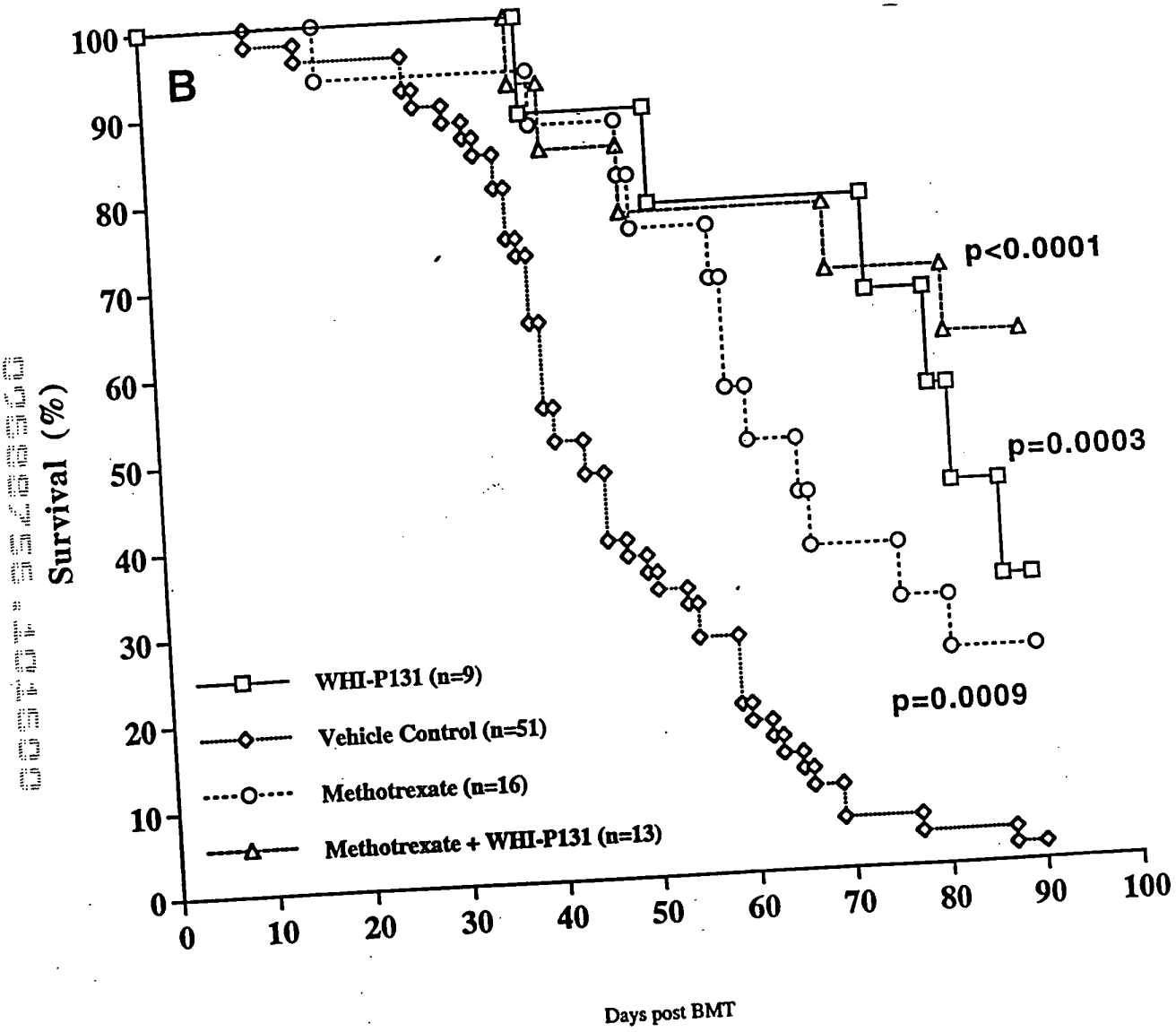

A
p=0.0003
Survival (%)
Days post BMT
WHI-P131 (n=9)
Vehicle Control (n=51)
Cyclosporin A (n=15)
Cyclosporin A + WHI-P131 (n=15)
0
10
20
30
40
50
60
70
80
90
100

Figure 25


B
Survival (%)
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
90
100
Days post BMT
WHI-P131 (n=9)
Vehicle Control (n=51)
Methotrexate (n=16)
Methotrexate + WHI-P131 (n=13)
p<0.0001
p=0.0003
p=0.0009

WHI-P131 (60 mg/kg/day) - effects on GVHD development

Group	n	Cumulative proportion surviving 30	Cumulative proportion surviving 60	Cumulative proportion surviving 90	median survival (d)	Logrank p value vs. Control	Logrank p value vs. WHI-P
CONTROL	51	90.2 ± 4.2	17.6 ± 5.3	2.0 ± 1.9	44		
WHI-P131	9	100 ± 0	77.8 ± 13.9	33.3 ± 15.7	82	0.0003	
WHI-P132	9	88.9 ± 10.5	11.1 ± 10.5	0 ± 0	44	0.485	
Methotrexate	16	93.8 ± 6.1	56.2 ± 12.4	25.0 ± 10.8	63.5	0.0009	0.356
Methotrexate + WHI-P131	13	100 ± 0	76.9 ± 11.7	61.5 ± 13.5	87	<0.0001	0.320
Cyclosporine A	15	73.3 ± 11.4	26.7 ± 11.4	13.3 ± 8.8	45	0.493	0.029
Cyclosporine A + WHI-P131	15	80.0 ± 10.3	33.3 ± 12.2	20.0 ± 10.3	51	0.198	0.06[illegible]

Methotrexate vs.
Methotrexate + WHI-P131
0.062

26

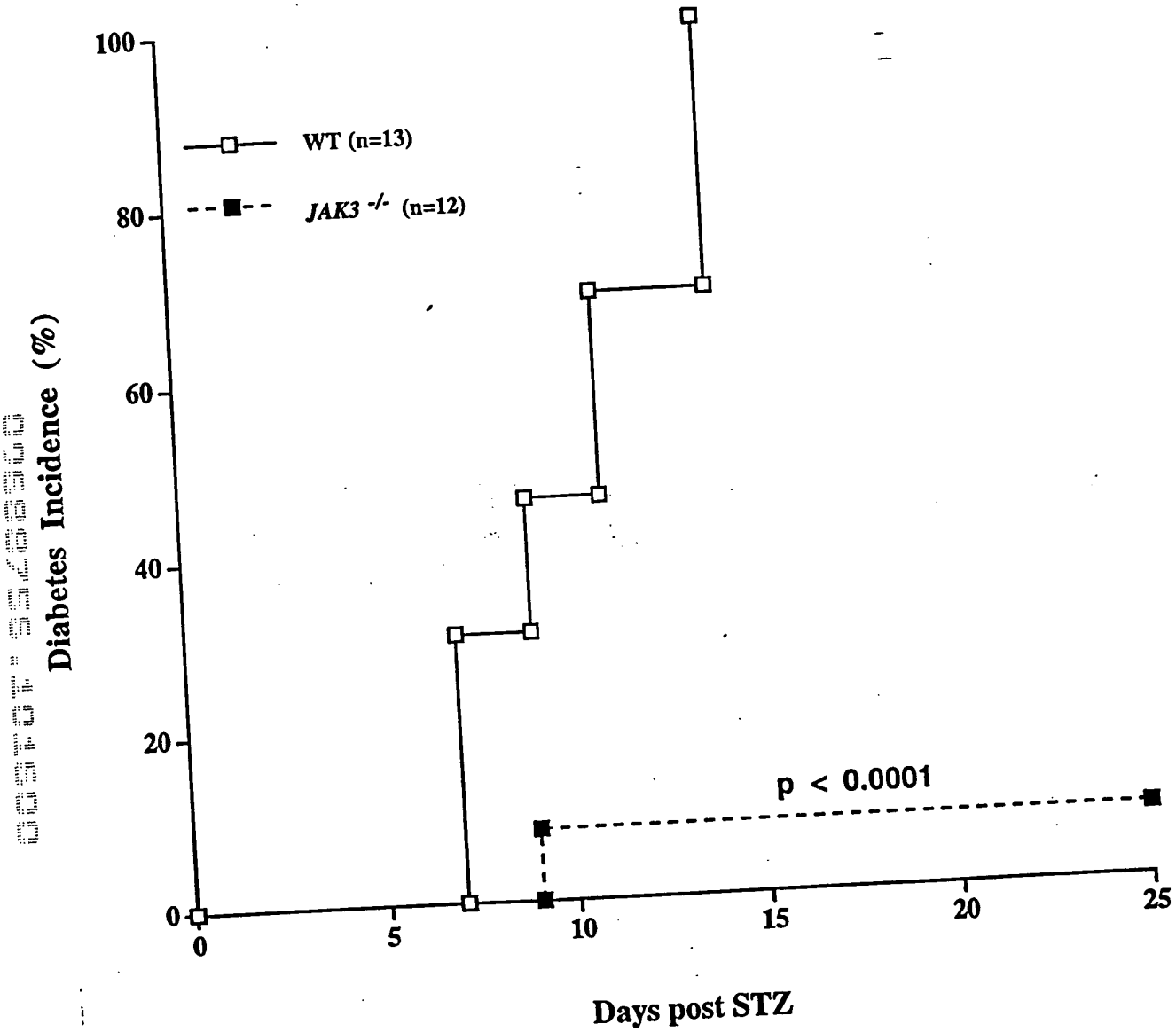

100
80
60
40
20
0
0
5
10
15
20
25
WT (n=13)
JAK3 -/- (n=12)
p < 0.0001
Diabetes Incidence (%)
Days post STZ

Figure 28

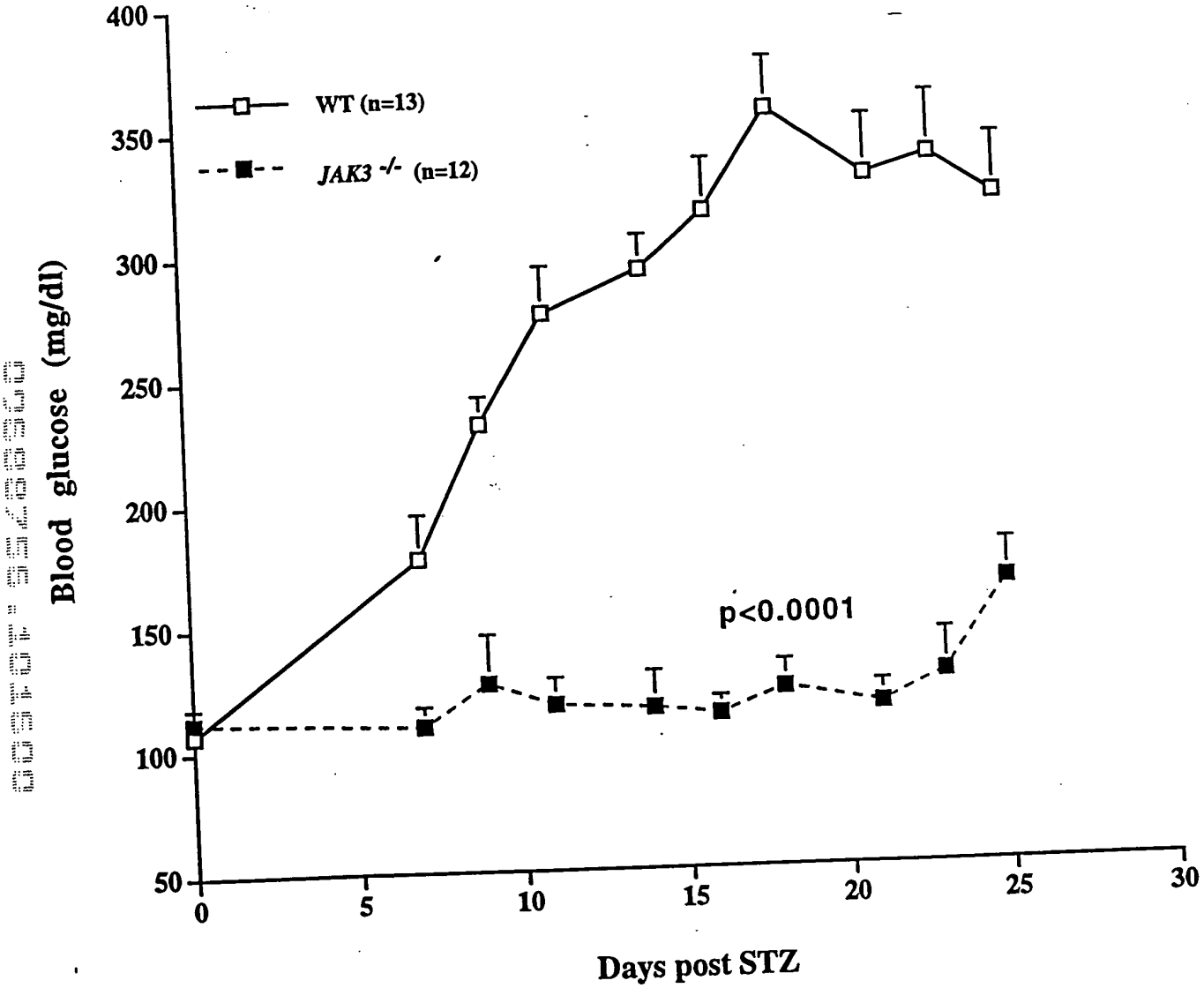

400
350
300
250
200
150
100
50
WT (n=13)
JAK3 -/- (n=12)
p<0.0001
Blood glucose (mg/dl)
0
5
10
15
20
25
30
Days post STZ

Figure 29

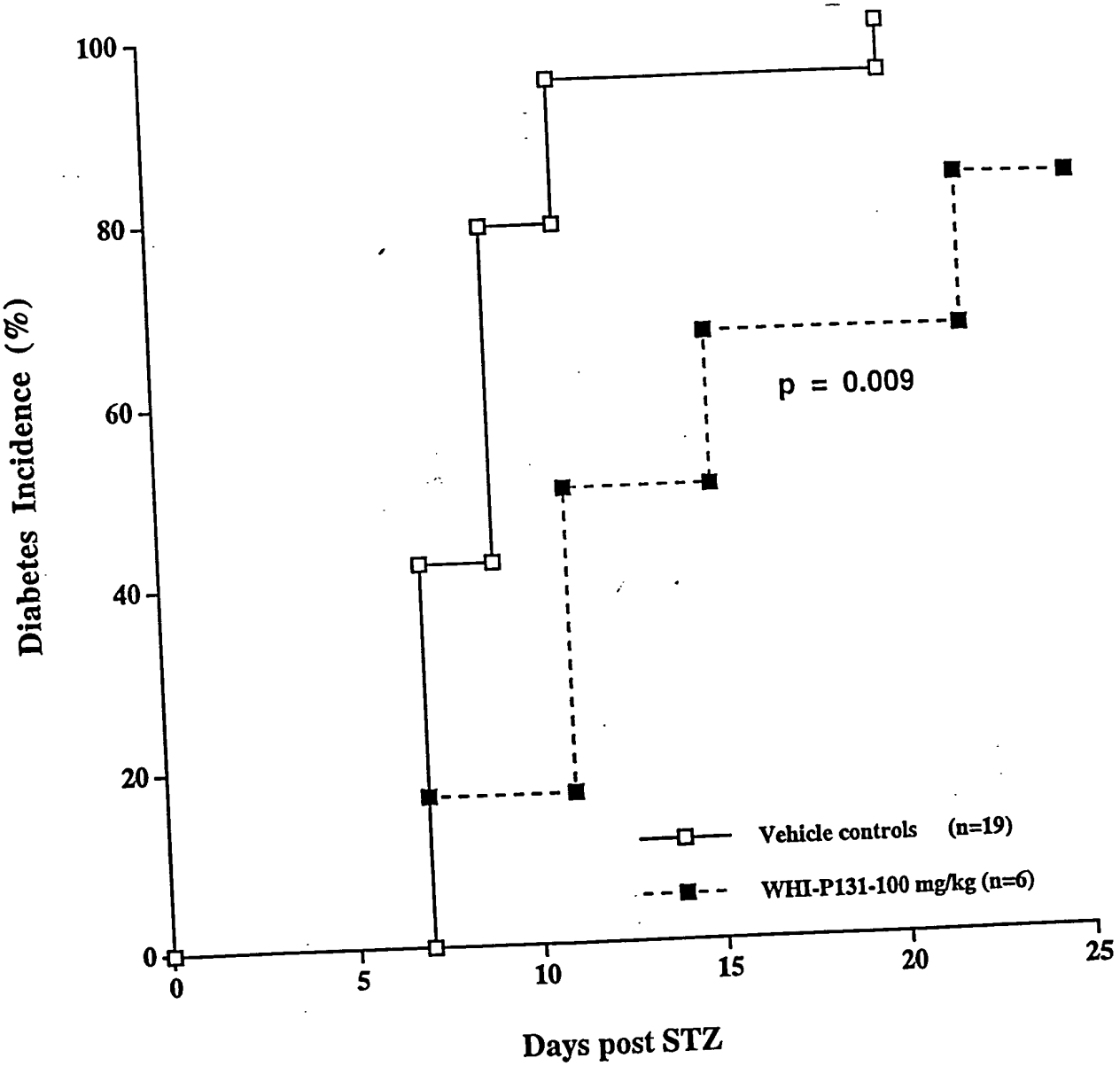

Diabetes Incidence (%)
100
80
60
40
20
0
0
5
10
15
20
25
Days post STZ
p = 0.009
Vehicle controls (n=19)
WHI-P131-100 mg/kg (n=6)

Figure 30

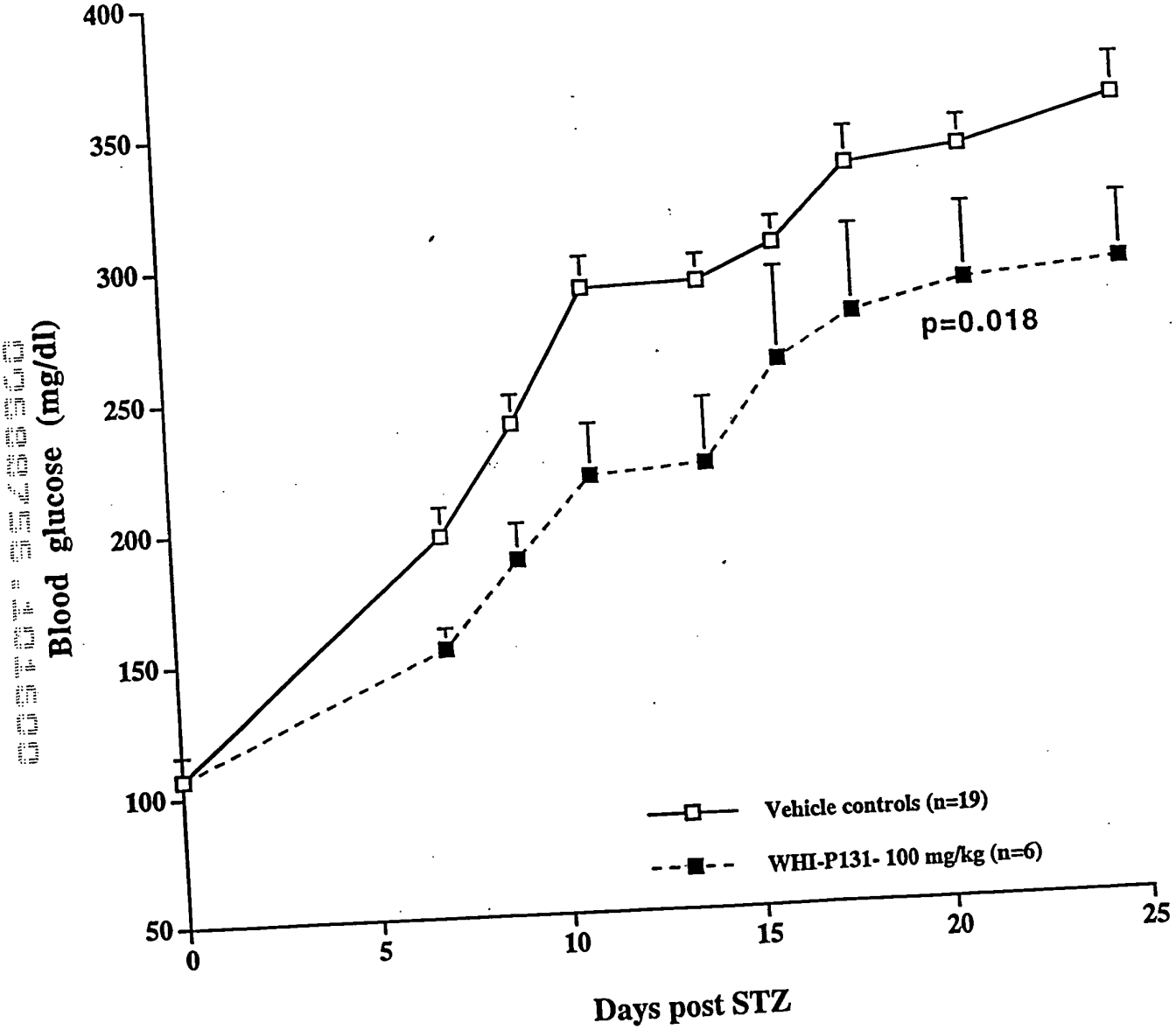

400
350
300
250
200
150
100
50
0
5
10
15
20
25
Blood glucose (mg/dl)
Days post STZ
p=0.018
Vehicle controls (n=19)
WHI-P131- 100 mg/kg (n=6)

Figure 31

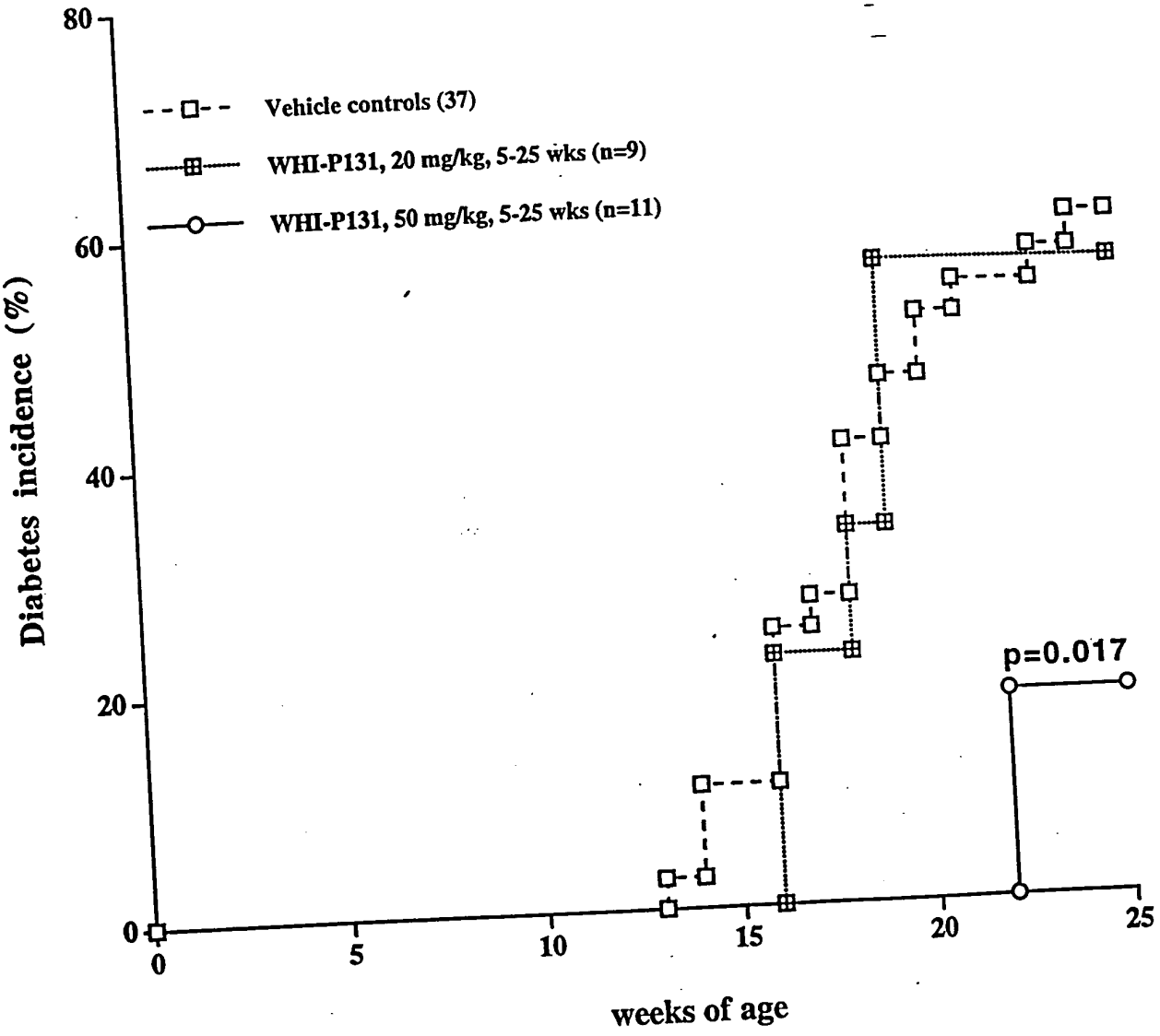

Vehicle controls (37)
WHI-P131, 20 mg/kg, 5-25 wks (n=9)
WHI-P131, 50 mg/kg, 5-25 wks (n=11)
Diabetes incidence (%)
80
60
40
20
0
0
5
10
15
20
25
weeks of age
p=0.017

Figure 32

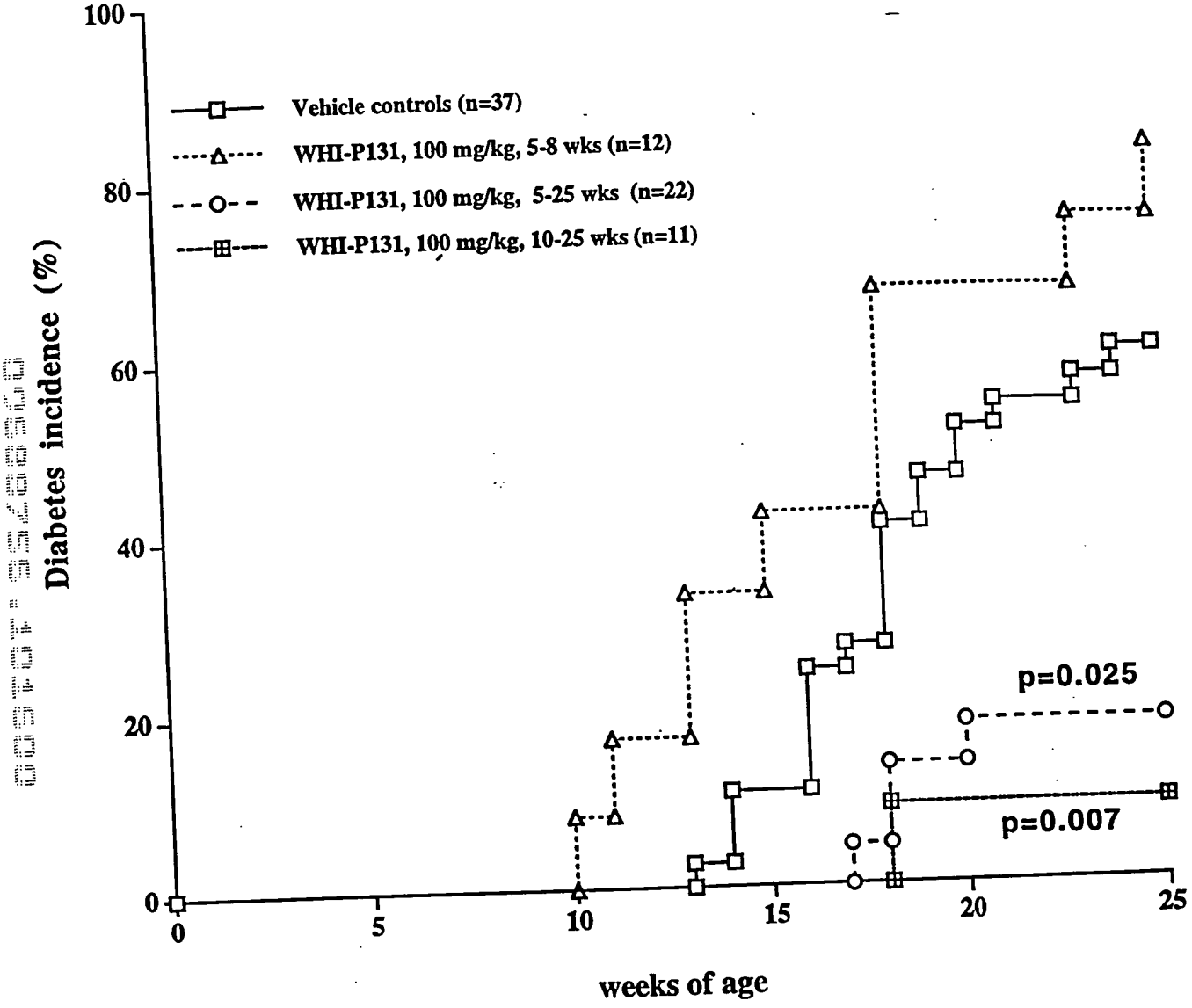

Vehicle controls (n=37)
WHI-P131, 100 mg/kg, 5-8 wks (n=12)
WHI-P131, 100 mg/kg, 5-25 wks (n=22)
WHI-P131, 100 mg/kg, 10-25 wks (n=11)
Diabetes incidence (%)
100
80
60
40
20
0
0
5
10
15
20
25
weeks of age
p=0.025
p=0.007

Figure 33

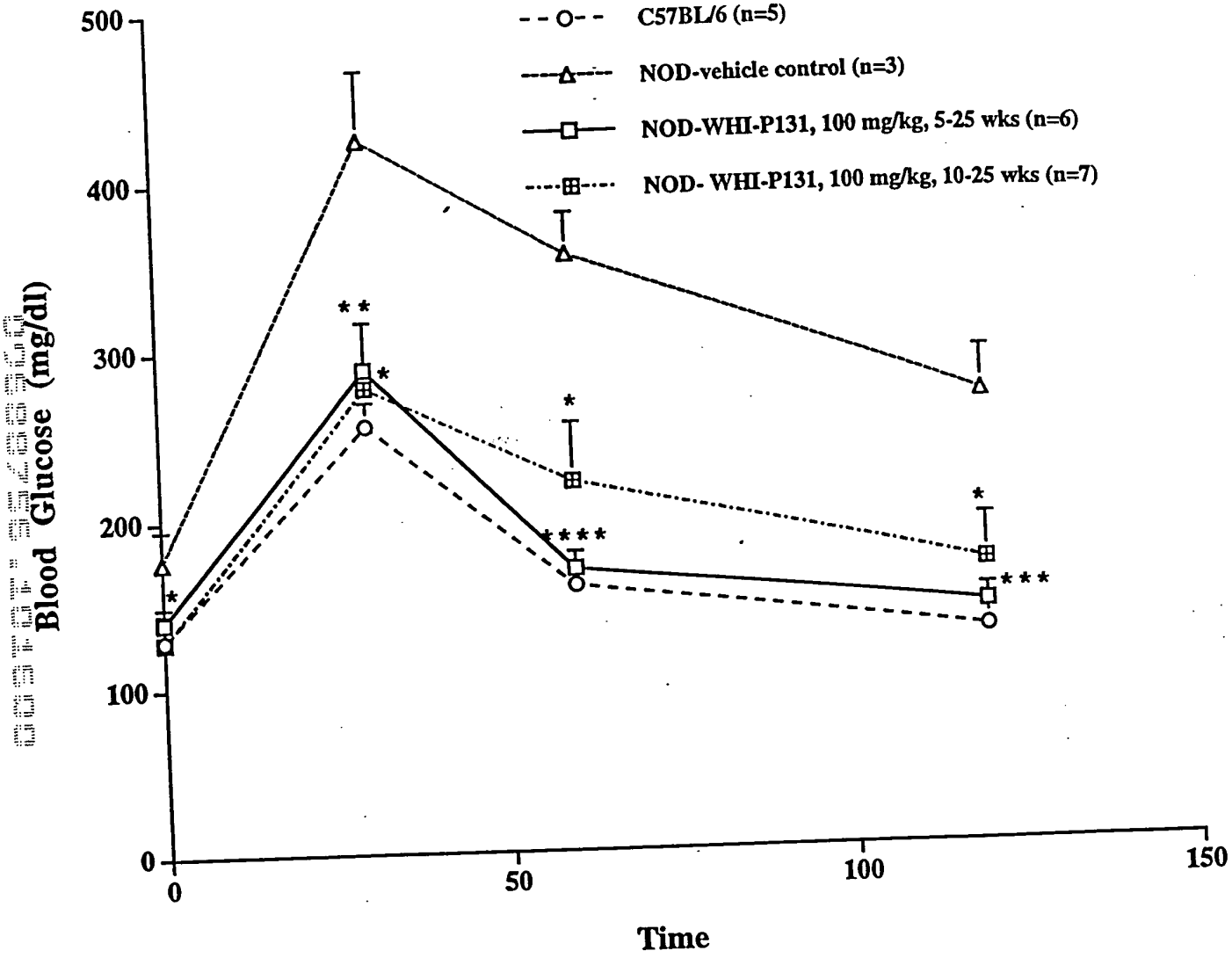

C57BL/6 (n=5)
NOD-vehicle control (n=3)
NOD-WHI-P131, 100 mg/kg, 5-25 wks (n=6)
NOD- WHI-P131, 100 mg/kg, 10-25 wks (n=7)
Blood Glucose (mg/dl)
500
400
300
200
100
0
0
50
100
150
Time

Figure 27

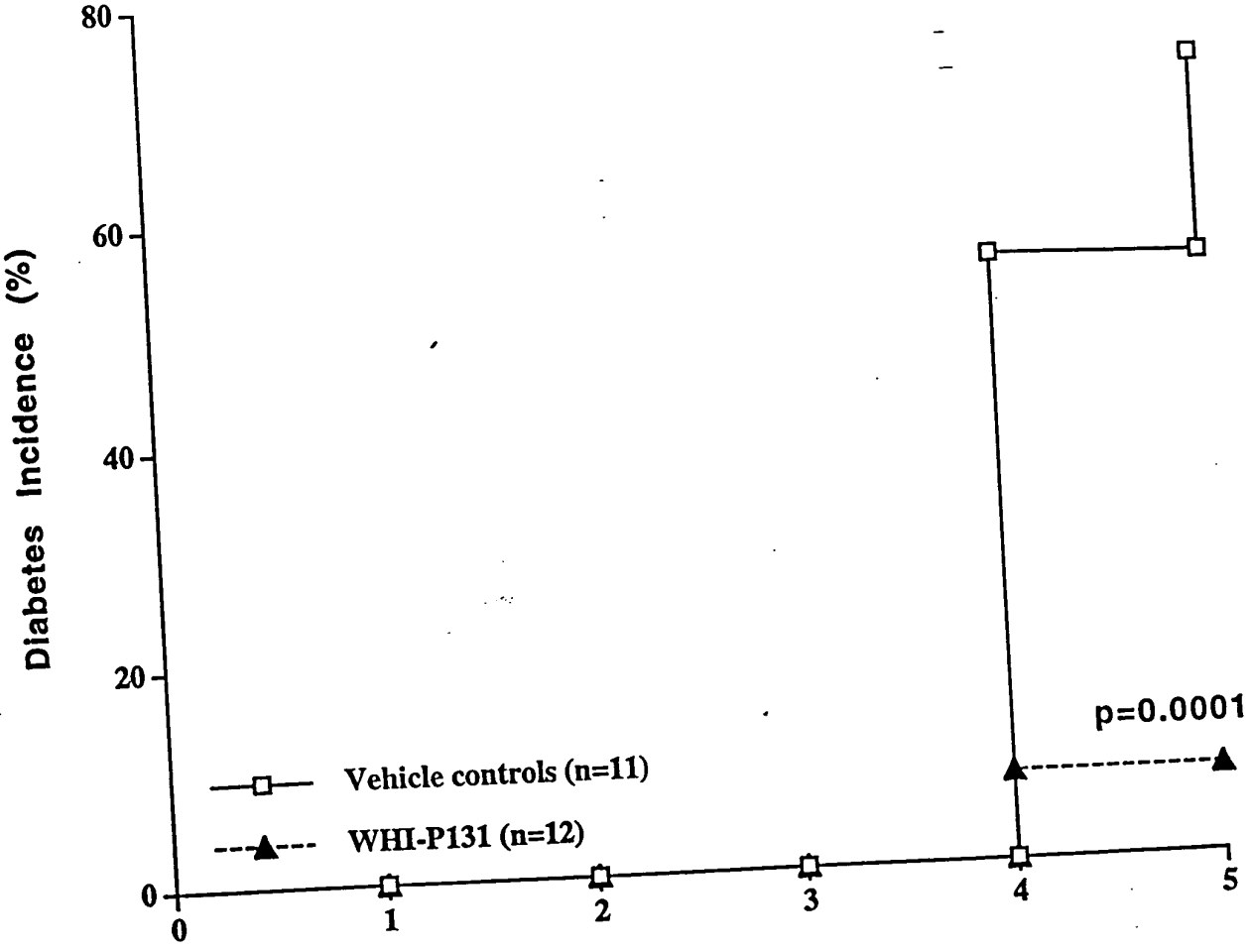

Diabetes Incidence (%)
80
60
40
20
0
0
1
2
3
4
5
p=0.0001
Vehicle controls (n=11)
WHI-P131 (n=12)

Figure 35

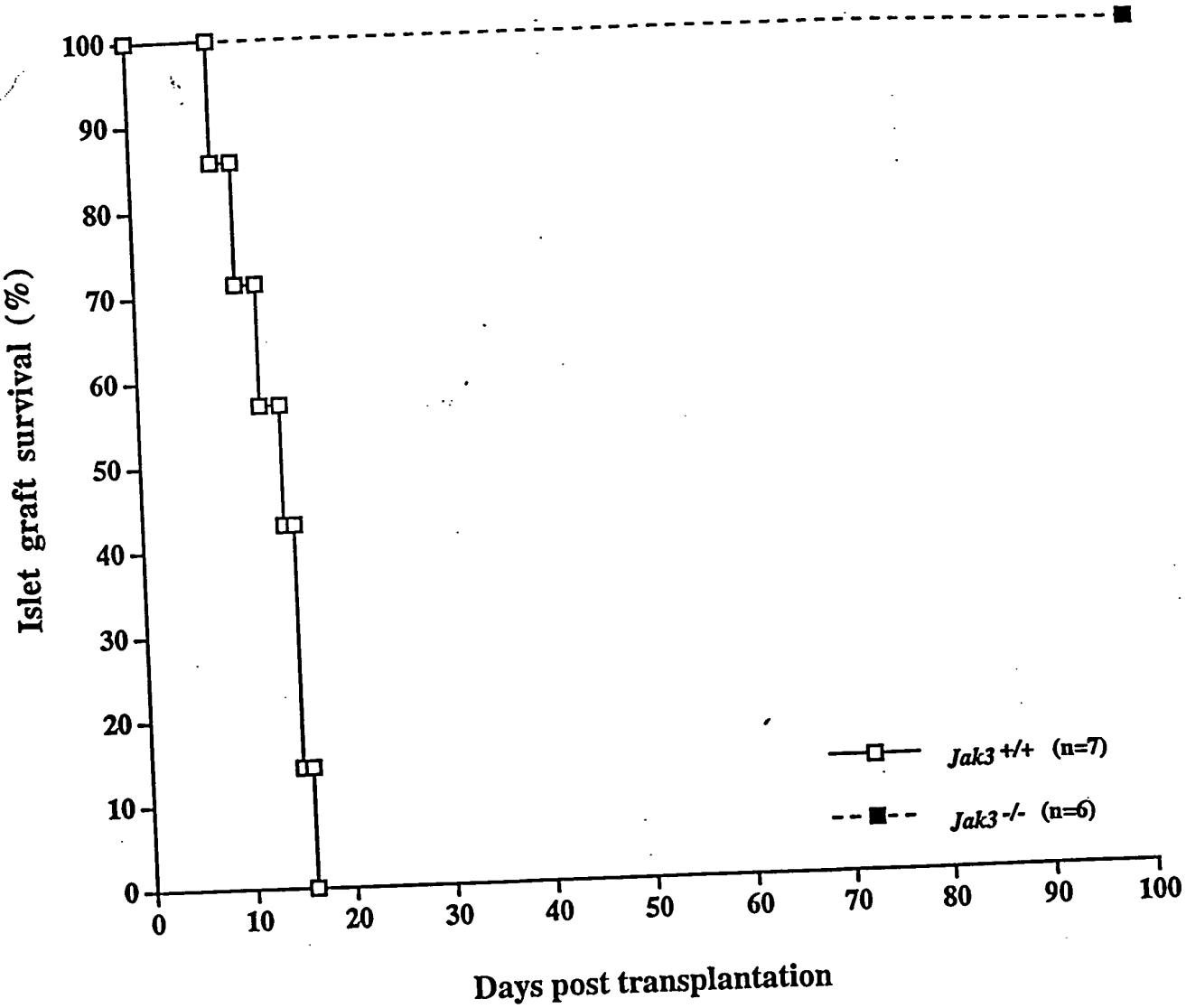

Islet graft survival (%)
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
90
100
Days post transplantation
Jak3 +/+ (n=7)
Jak3 -/- (n=6)

Figure 36

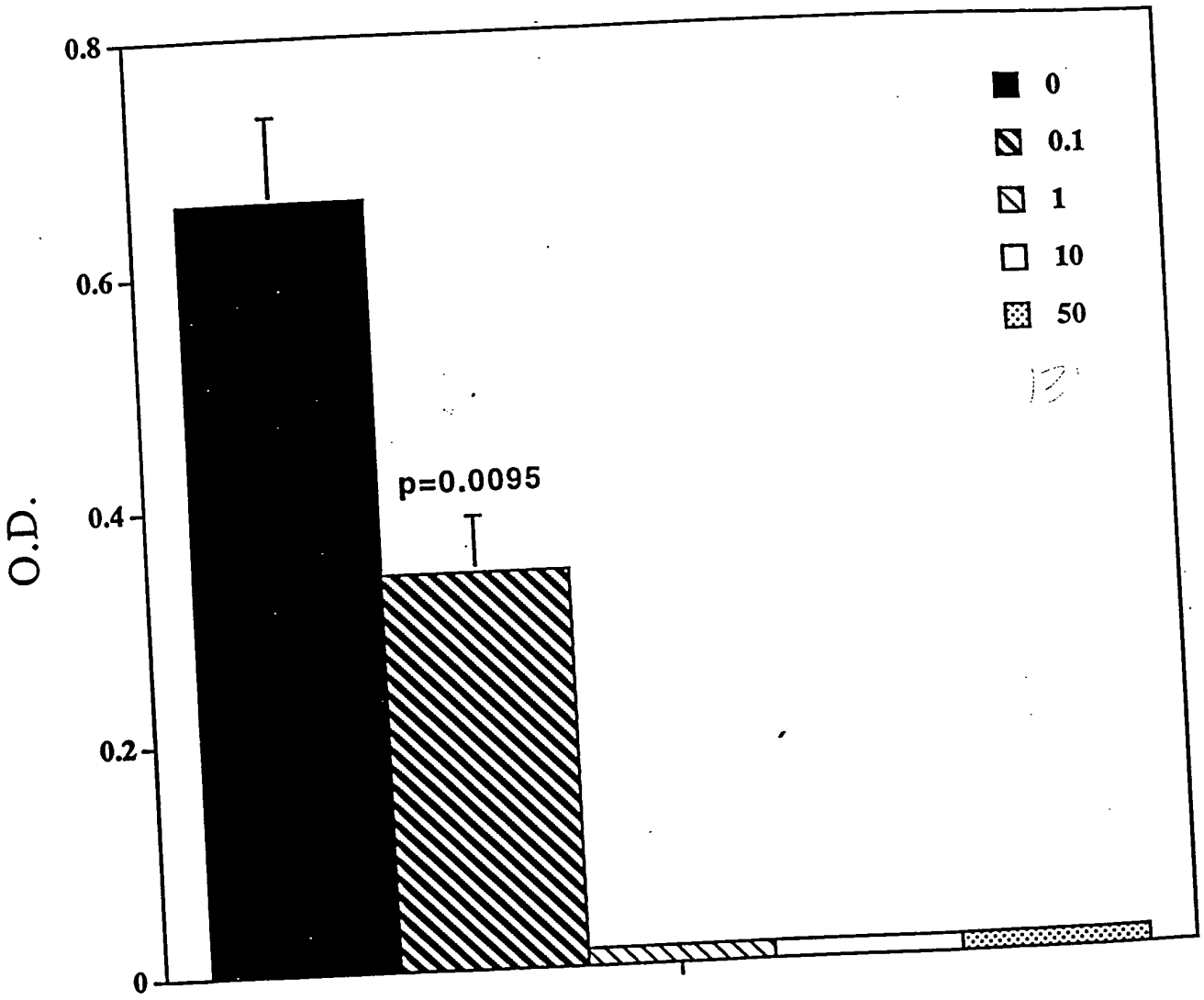


Figure 5I

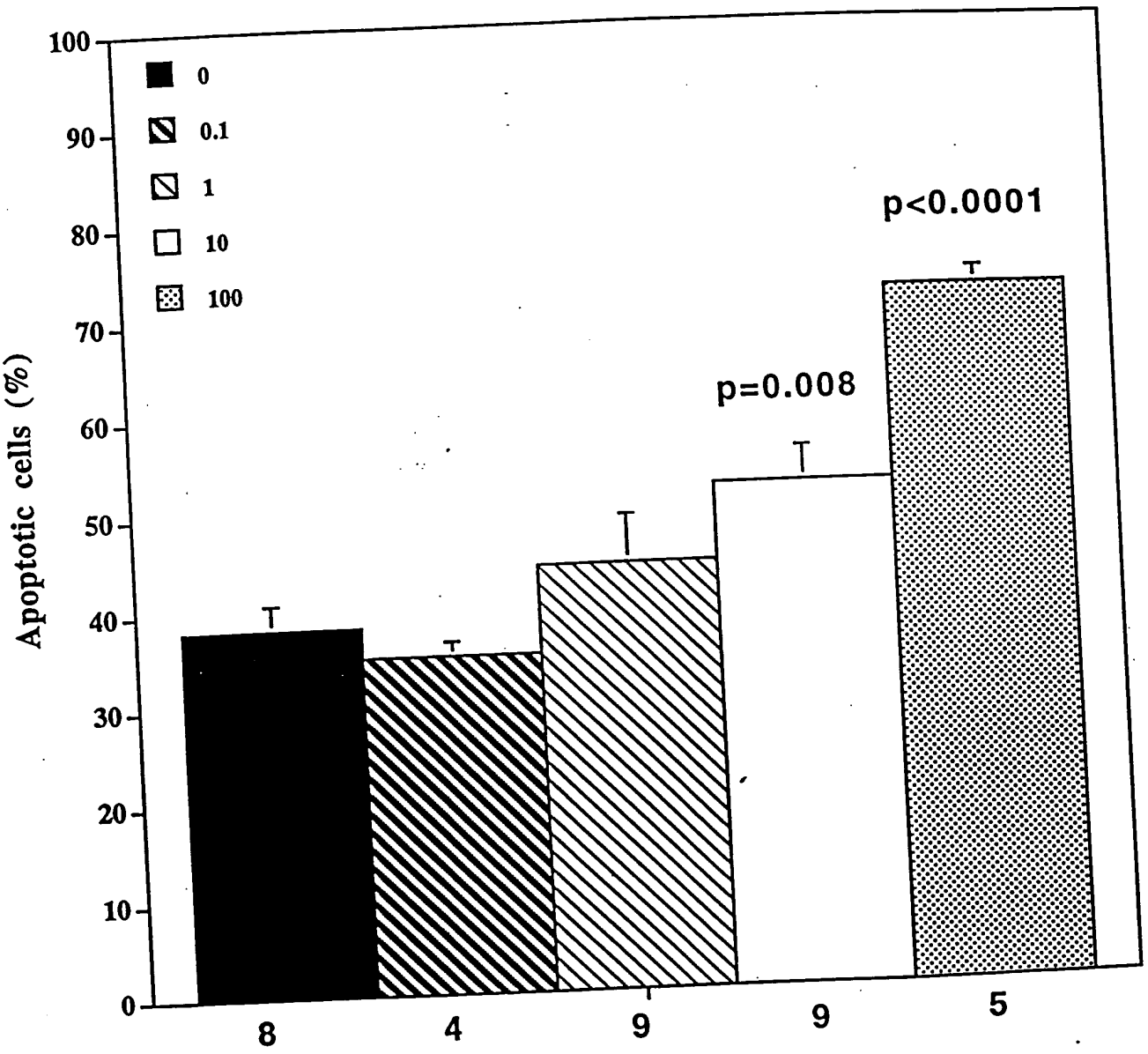

100
90
80
70
60
50
40
30
20
10
0
Apoptotic cells (%)
0
0.1
1
10
100
p<0.0001
p=0.008
8
4
9
9
5

Figure 30

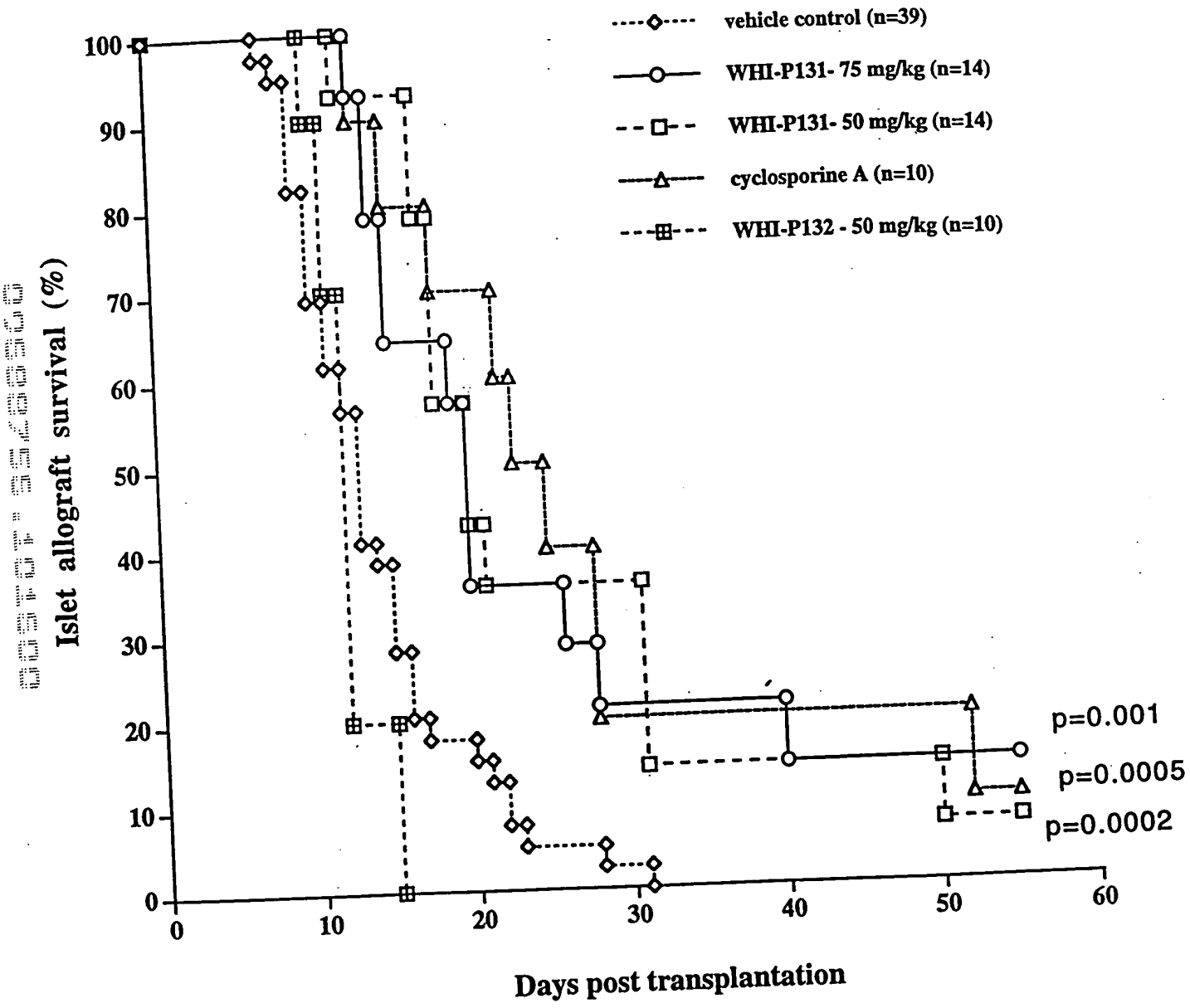

vehicle control (n=39)
WHI-P131- 75 mg/kg (n=14)
WHI-P131- 50 mg/kg (n=14)
cyclosporine A (n=10)
WHI-P132 - 50 mg/kg (n=10)
Islet allograft survival (%)
Days post transplantation
p=0.001
p=0.0005
p=0.0002
0
10
20
30
40
50
60
70
80
90
100

Figure 31

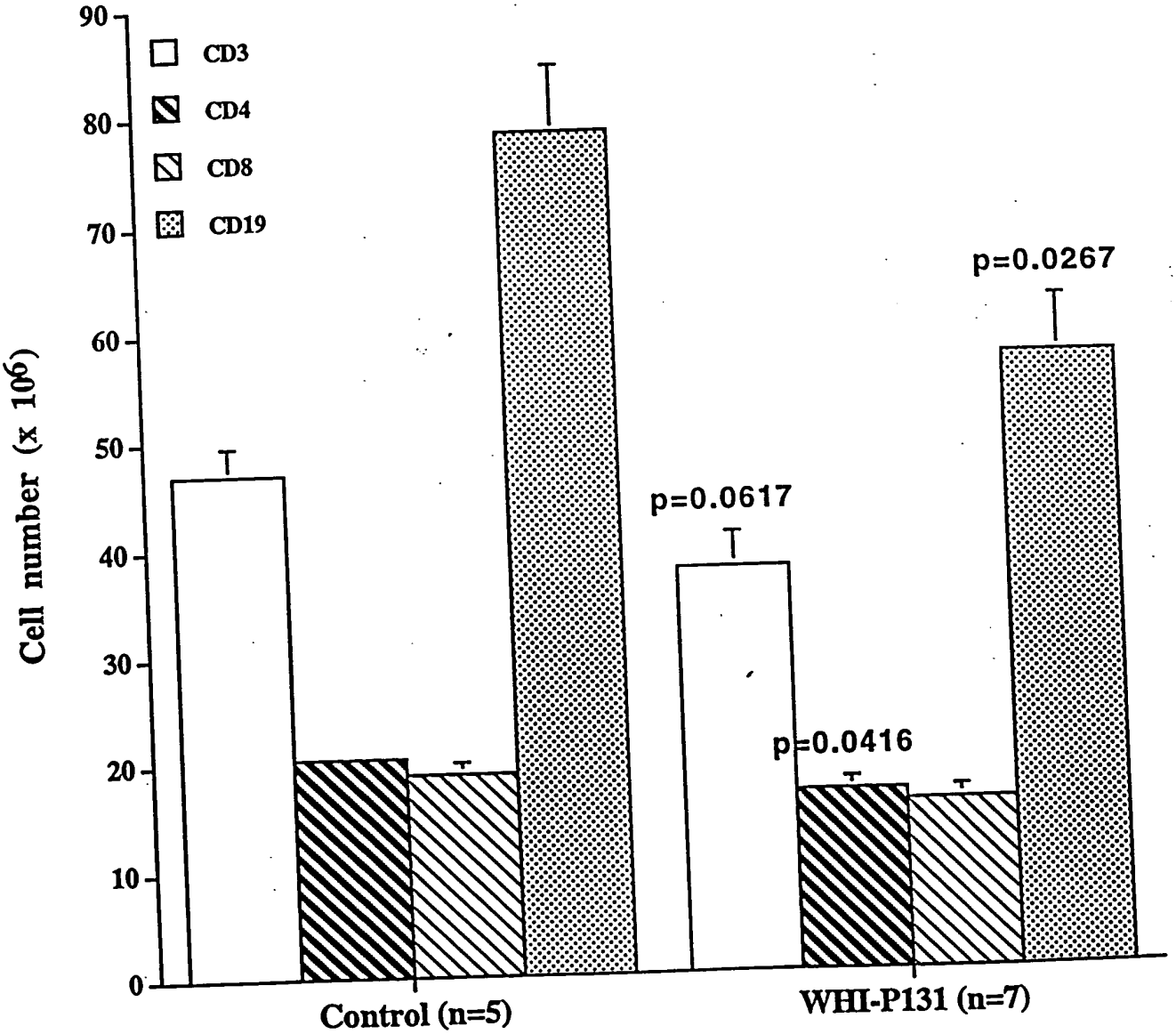

90
80
70
60
50
40
30
20
10
0
Cell number (x 10⁶)
CD3
CD4
CD8
CD19
p=0.0617
p=0.0416
p=0.0267
Control (n=5)
WHI-P131 (n=7)

Figure 40

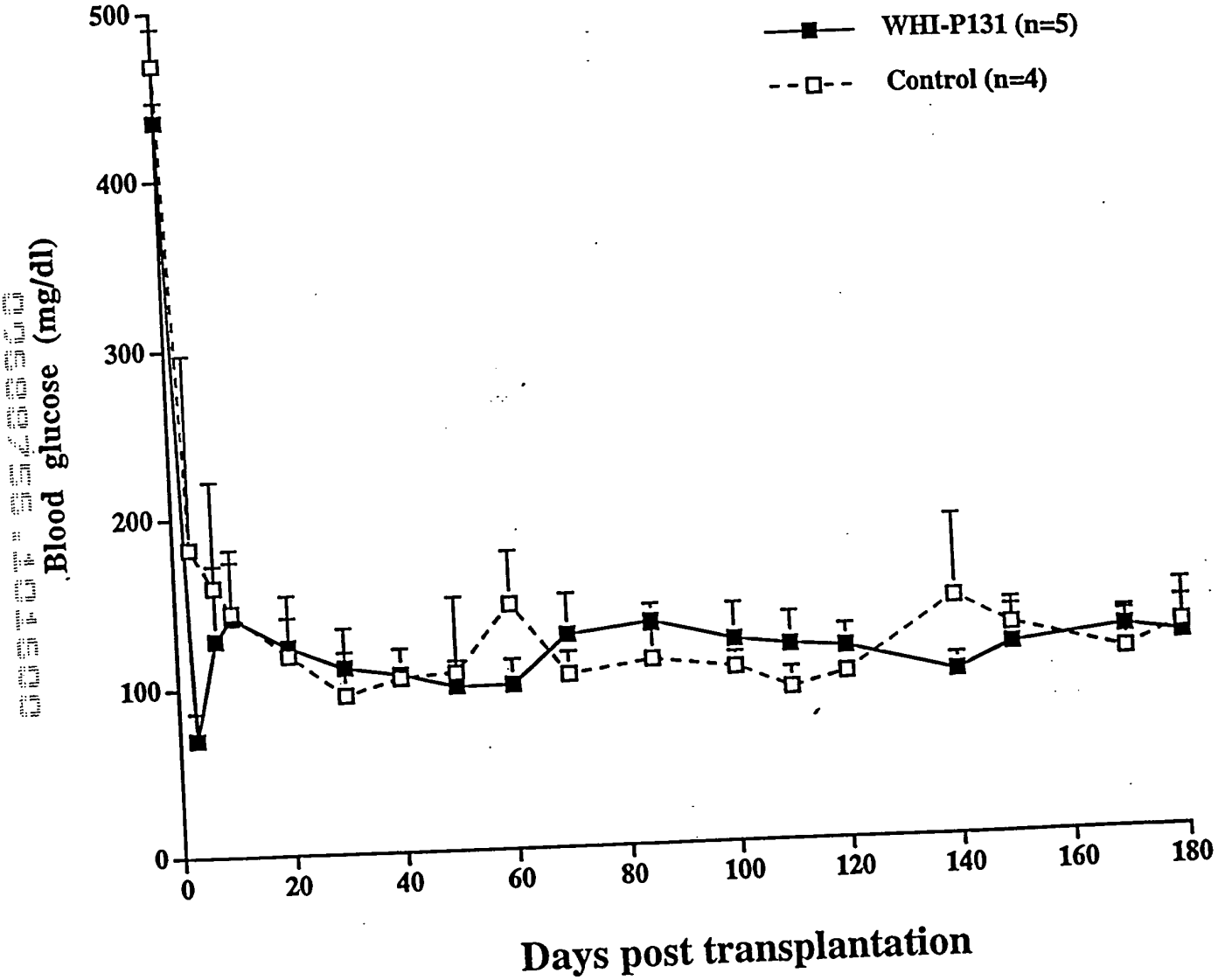
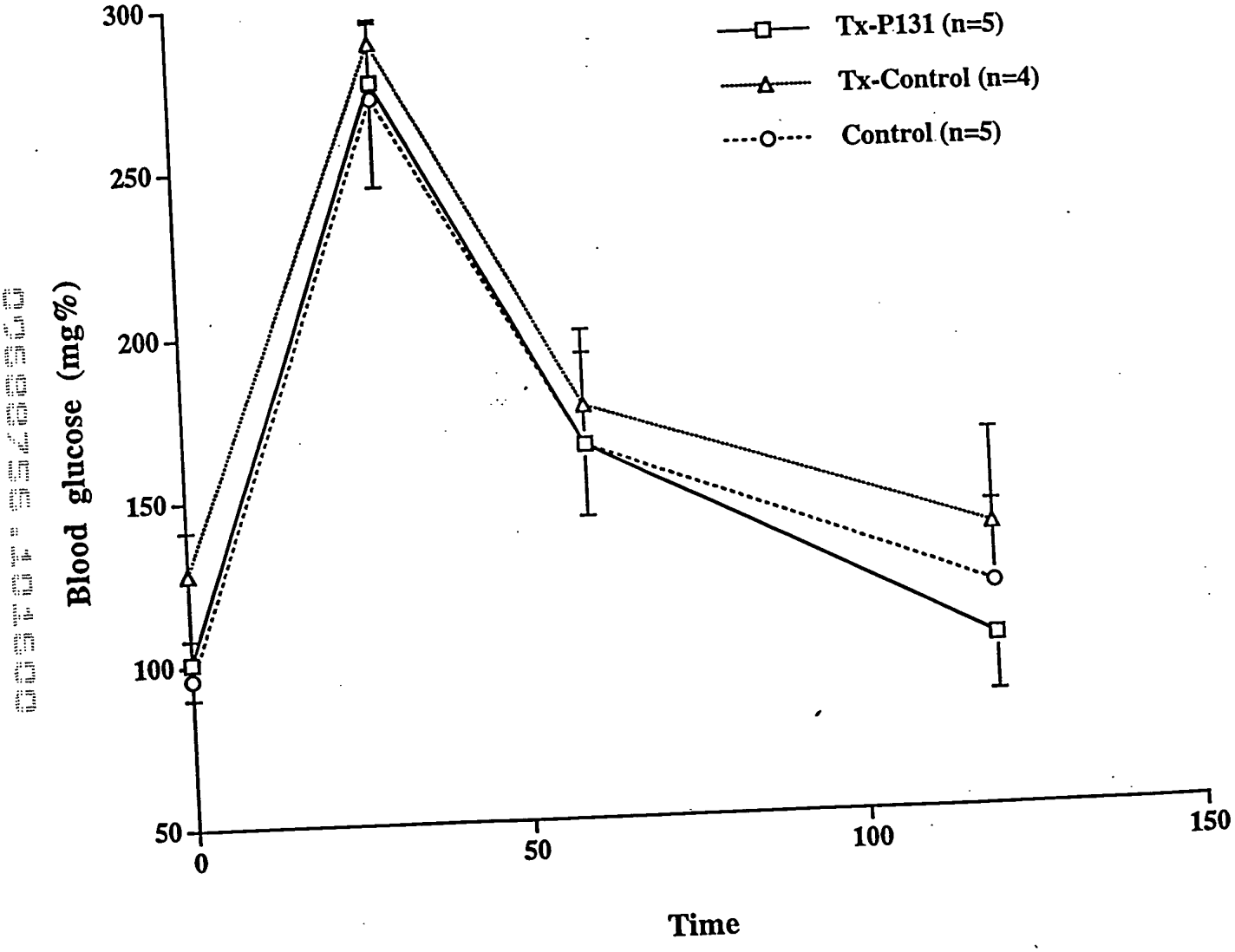
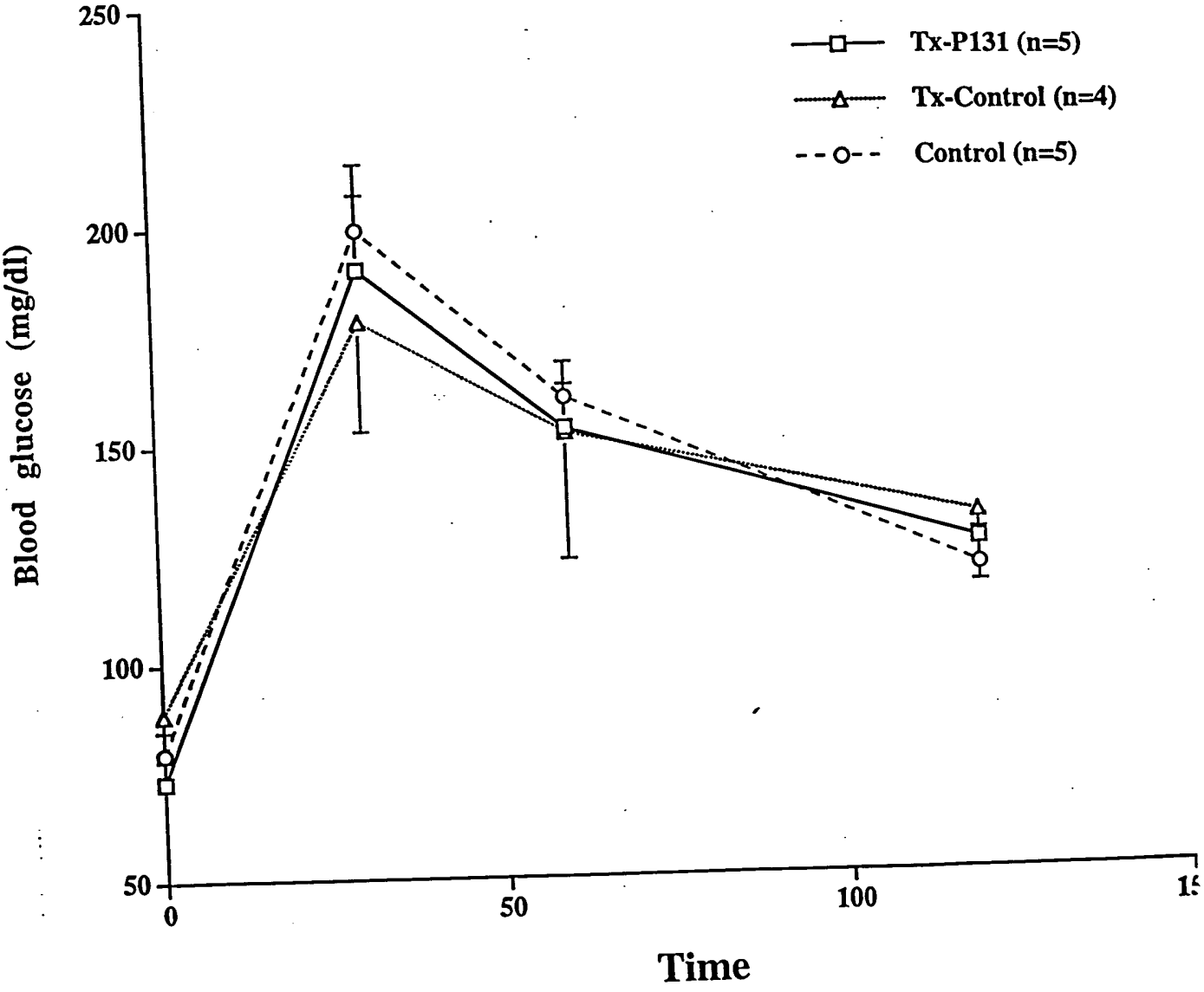

WHI-P131 (n=5)
Control (n=4)
Blood glucose (mg/dl)
500
400
300
200
100
0
0
20
40
60
80
100
120
140
160
180
Days post transplantation

Figure 71


Tx-P131 (n=5)
Tx-Control (n=4)
Control (n=5)
Blood glucose (mg%)
300
250
200
150
100
50
0
50
100
150
Time


Tx-P131 (n=5)
Tx-Control (n=4)
Control (n=5)
Blood glucose (mg/dl)
250
200
150
100
50
0
50
100
Time

42